MISCELLANEOUS PAPER EL-90-11

REGULATORY EVALUATION OF PETROLEUM HYDROCARBONS IN DREDGED MATERIAL

PROCEEDINGS OF A WORKSHOP

Compiled by

Joan U. Clarke, A. Susan Jarvis

Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
3909 Halls Ferry Road, Vicksburg, Mississippi 39180-6199

July 1990

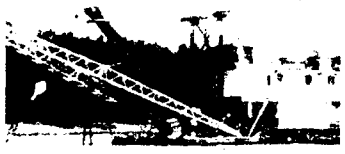
Final Report

Approved for Public Release; Distribution Unlimited

Prepared for US Army Engineer District, Chicago
Chicago, Illinois 60604-1797

and

US Army Engineer District, New York
New York, New York 10278-0090



Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Miscellaneous Paper EL-90-11	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION USAEWES Environmental Laboratory 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 3909 Halls Ferry Road Vicksburg, MS 39180-6199	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION USAED, Chicago and New York 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Chicago, IL 60604-1797; New York, NY 10278-0090	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) Regulatory Evaluation of Petroleum Hydrocarbons in Dredged Material; Proceedings of a Workshop	
12. PERSONAL AUTHOR(S) Clarke, Joan U.; Jarvis, A. Susan	
13a. TYPE OF REPORT Final report 13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day) July 1990 15. PAGE COUNT 137
16. SUPPLEMENTARY NOTATION Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.	
17. COSATI CODES FIELD / GROUP / SUB-GROUP	18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) See reverse.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A 3-day workshop on the regulatory interpretation of petroleum hydrocarbons in dredged material was conducted ~~15-17~~ March ~~1988~~ at the US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. The workshop was held at the request of US Army Engineer Districts, Chicago and New York, and followed an earlier (1986) workshop on regulatory evaluation of petroleum hydrocarbons in dredged material. This report is a detailed summary of the second workshop proceedings.

Workshop participants, representing government agencies, private industry, and academia, were selected for their expertise in environmental chemistry and biological effects of polycyclic aromatic hydrocarbons (PAH). The primary objective of the workshop

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20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code) / 22c. OFFICE SYMBOL

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

Unclassified

18. SUBJECT TERMS (Continued).

Acute toxicity tests
Bioaccumulation tests
Bioavailability
Dredged material
Petroleum hydrocarbons
Polycyclic aromatic hydrocarbons (PAH)
Priority pollutant PAH
QA/QC
Regulatory evaluation
Sediment analysis
Tiered testing

19. ABSTRACT (Continued).

was to develop guidance on scientific interpretation of potential impacts of PAH. Prior to the workshop, participants were asked to submit written answers to specific questions in a provided questionnaire. The questionnaire was divided into three sections: (1) a reexamination of the recommendations of the 1986 PAH workshop, (2) sediment analyses and biological testing for PAH, and (3) the biological effects of PAH.

Roundtable discussions centered on the workshop objectives, questionnaire responses, and other issues raised by the District sponsors. The 1986 PAH workshop participants had recommended a list of 15 priority pollutant PAH for regulatory analysis of dredged material and also recommended a two-tiered testing approach consisting of first-tier acute toxicity tests and sediment analysis for the 15 PAH, and second-tier 10-day bioaccumulation tests. Participants in the second workshop generally agreed that the list of 15 PAH selected in the first workshop should remain unchanged. The tiered testing approach was expanded from two to four tiers, with each tier based upon a "reason to believe" that there is potential for unacceptable adverse biological effects, in conformance with the Federal Standard. Tier I is the initial determination of "reason to believe" that the sediment is contaminated with PAH. Tier II specifies chemical analysis of the sediment for the 15 selected PAH to assess whether the dredged material is more contaminated than the disposal site or an appropriate reference sediment, at least in terms of "total PAH" as the sum of the 15. Tier III is the first biological testing tier, which includes acute toxicity tests and bioaccumulation tests. Recommendations of specific animals to use in different test situations were made. Tier IV is the sublethal effects assessment, but tests for this evaluation are not sufficiently developed or verified for implementation in regulatory programs at the present time.

This testing approach should not be considered the final answer to regulatory evaluation of PAH-contaminated dredged material, but only as a direction in which Corps Districts may proceed for the present. Considerably more research and information are needed to develop a detailed, comprehensive testing approach for PAH in sediment. Specifically, a database needs to be developed on environmental levels and biological effects of the 15 PAH with the objective of establishing technically defensible, effects-based regulatory criteria for PAH.

SUMMARY

This report summarizes proceedings of the second petroleum hydrocarbons workshop, held 15-17 March 1988 at the US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. The workshop was held in response to a request by the US Army Engineer Districts, Chicago and New York, for assistance in regulatory evaluation of petroleum hydrocarbons in dredged material. Scientists known for their expertise in the environmental chemistry and biological effects of petroleum hydrocarbons were invited to participate in the workshop.

The first petroleum hydrocarbons workshop, held in 1986, was sponsored by the New York District. That workshop focused on identification of specific petroleum hydrocarbons that would be appropriate for analysis as a basis for regulation of dredged material disposal. The workshop participants agreed that the polycyclic aromatic hydrocarbons (PAH) are the most important class of petroleum hydrocarbons in dredged material due to their toxicity and persistence. Recommendations were made for chemical analysis of 15 "priority pollutant" PAH[1] and biological testing in a tiered testing approach. Results of the workshop (hereafter referred to as the 1986 PAH workshop) were summarized by Clarke and Gibson (1987a,b) and Clarke (1987). The summary of major agreements from the 1986 PAH workshop can also be found in Part I of this report.

The second workshop built upon the recommendations of the 1986 PAH workshop, and emphasized the interpretation of the recommended PAH testing approach results. This workshop was sponsored by the Chicago District, but like the first workshop, was intended to address the divergent concerns and regulatory requirements of both the Chicago and New York Districts, and also to provide general technical guidance to all Corps of Engineers Districts concerning the regulatory evaluation of dredged material containing PAH. The resulting guidance incorporates aspects of both the chemistry-based assessment traditionally applied in the Great Lakes under the Clean Water Act for inland and nearshore disposal, and the biological effects-based assessment required under the Ocean Dumping Act for coastal and offshore ocean disposal. The actual guidance proposed at the workshop has been modified slightly to conform

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1. Priority pollutants refer to a list of 129 toxic substances compiled by the US Environmental Protection Agency (USEPA). The list includes 16 PAH.

to the national comprehensive testing strategy supported by the Corps (Engler et al. 1988).

The workshop participants reexamined the list of 15 PAH selected during the 1986 workshop for regulatory evaluation of dredged material, and generally agreed that the list should remain unchanged. These PAH are acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, phenanthrene, and pyrene; they include 15 of the 16 priority pollutant PAH. Minority opinion favored the addition of the 16th priority pollutant PAH, naphthalene, to the list, but organic chemists in the group maintained that there are potentially serious problems in obtaining accurate chemical analysis of naphthalene. Other suggested additions to the list, such as methyl-substituted naphthalenes and other alkyl-substituted PAH, and benzo[e]pyrene, were rejected at this time because of analytical problems, because of similarity in structure and effects with PAH already on the list, or because not enough is yet known about their behavior and biological effects in sediment.

PAH have been associated with a number of acute and chronic toxic effects in organisms, including mortality, impairment of growth and reproductive processes, and carcinogenicity. Acute toxicity from PAH in sediment would most likely occur in sensitive, sediment-dwelling animals such as polychaetes, amphipods, or insect larvae. Chronic or sublethal effects generally result from biotransformation of the parent PAH compounds to more toxic metabolites. These effects would tend to occur in organisms having well-developed biotransformation capability for PAH, such as fishes and some invertebrates. Molluscs, particularly bivalves, are thought to have limited biotransformation capability for PAH, and thus can be good indicators of PAH bioavailability because they will accumulate parent PAH compounds in their tissues. Bioavailability of PAH is important as a prerequisite for contaminant-related biological impact.

Both USAE District sponsors requested effects-based numeric guidelines for PAH in sediment or tissues, to be used in their regulatory programs. The Chicago District sought upper and lower sediment PAH thresholds that could be used to identify sediment that would not require biological testing. For example, sediment having PAH concentrations below the lower thresholds could be considered uncontaminated (at least in terms of PAH) and a candidate for unrestricted aquatic disposal. Conversely, sediment having PAH concentrations

above the upper thresholds could be considered "grossly" contaminated and unsuitable for unrestricted aquatic disposal. Sediment with PAH concentrations falling between the thresholds would require biological testing before regulatory decisions could be made. The New York District sought similar numeric guidelines for PAH in tissues.

It became increasingly clear during the workshop that the desire of the Districts for numeric PAH regulatory guidelines could not be achieved. There are no current "levels of concern" for PAH, because there is simply not enough information at present to unequivocally and quantitatively link adverse biological effects with concentrations of PAH either in sediment or in tissues. Moreover, the many complex factors influencing bioavailability and toxicity of PAH are poorly understood. For these reasons, scientifically sound regulatory evaluation of PAH-contaminated dredged material *must be based on bioassessment* rather than on numeric criteria.

The first assessment for regulatory evaluation of dredged material must be the determination of whether there is reason to believe that the dredged material is contaminated with PAH. This assessment could be based upon historic data, knowledge of point sources or impacts such as spills, or any other relevant information. If a "reason to believe" exists, then the regulatory evaluation would proceed with chemical and biological testing for PAH. If there is no "reason to believe," then such testing would not be necessary for PAH.

A good regulatory program for PAH-contaminated dredged material should incorporate a suite of biological tests to assess the bioavailability of the PAH and the potential adverse effects that disposal of the dredged material might have on aquatic organisms. The tests (and organisms) used should be sensitive to the contaminants in the dredged material to be regulated, and site specific to the extent that they assess the particular impacts known or suspected to occur in the dredging and disposal areas. However, the tests cannot be contaminant specific because there are hundreds of compounds in sediment that can potentially cause adverse effects. All tests should be standardized, and capable of producing reliable and accurate results when conducted by commercial (contract) laboratories.

Mortality due to acutely toxic compounds in sediment, such as high concentrations of some of the lower molecular weight PAH, may be assessed using acute toxicity tests with sensitive organisms. PAH bioavailability is best assessed using bioaccumulation tests that employ deposit-feeding organisms

having little ability to biotransform PAH. Chemical analysis of sediment for the 15 selected PAH is important to assess degree of contamination in the dredged material compared to that of the disposal site or appropriate reference site, and also to assist in the interpretation of the biological test results. These tests and analyses were all incorporated into a tiered testing approach recommended for regulatory evaluation of dredged material containing PAH. In addition, tests for sublethal effects of PAH in sediment are clearly needed, but have not been developed or evaluated to the extent that any specific test(s) can be recommended at this time for use in regulatory decision-making concerning sediment.

The recommended tiered testing approach for regulatory evaluation of PAH-contaminated dredged material has four tiers, in conformance with the comprehensive testing approach for dredged material disposal evaluation as part of the Federal Standard.[2] It incorporates, but goes beyond, the tiered testing scheme proposed at the 1986 PAH workshop. Engler et al. (1988) emphasized that each tier of the national comprehensive testing strategy is "based on a 'reason to believe' that there is potential for unacceptable adverse effects. Each tier is fully optional and may be subsequently eliminated if there is sufficient information available to provide an adequate assessment for that tier or if there is no reason to believe that there will be unacceptable adverse effects associated with that tier."

Tier I is the determination of "reason to believe" that the sediment is contaminated with PAH. Tier II specifies chemical analysis of the sediment for the 15 selected PAH to determine whether there is reason to believe that the dredged material is more contaminated than the disposal site (or reference) sediment, and that potential unacceptable adverse effects may occur. Tier III is the biological testing tier, including acute toxicity and bioaccumulation tests. The acute toxicity tests should use sensitive species; for water column or benthic environments these could include *Mysidopsis*, *Palaemonetes*, *Nereis*, *Rhepoxynius*, or *Ampelisca* in saltwater and *Daphnia*, *Ceriodaphnia*, *Selenastrum*, fathead minnows, *Chironomus*, or *Hexagenia* in freshwater. The bioaccumulation tests should use animals that have limited ability to me-

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2. The Federal Standard refers to the regulatory evaluation process intended to meet environmental requirements at the least cost within a consistent national framework (33 CFR Parts 209, 335, 336, 337, and 338, Discharge of Dredged Material Into Waters of the U.S. or Ocean Waters; Operation and Maintenance; Final Rule, Federal Register, 26 April 1988).

tabolize PAH, such as the bivalves *Macoma* or *Yoldia* for saltwater, the amphipod *Pontoporeia* for the Great Lakes, and perhaps another amphipod or *Hexagenia* for warmer freshwater environments. As in Tier II, the significance of Tier III results would be determined by comparing test results from dredging project sediment to results from the disposal site or an appropriate reference sediment. If the project test results are statistically significantly greater than the reference test results, then there is the potential for unacceptable adverse biological impacts to occur as a result of dredging and disposal operations.

At present there is little information relating concentrations of the 15 PAH in sediments or in tissues to biological effects, or assessing the relative toxicities of the 15 compounds. Thus it may be difficult to interpret Tier II and Tier III bioaccumulation results for the individual PAH. Until a database can be developed relating environmental concentrations of the individual PAH with biological effects, an interim recommendation would be to compare dredged material and reference test results for "total PAH" as a sum of the 15. Using this approach would have the advantage of generating values for the 15 individual PAH that could be incorporated into the database, but would not at present be used for regulatory decision making.

Tier IV, the sublethal effects assessment, might eventually specify an evaluation of the potential for adverse impact on reproduction and growth, perhaps using a partial life cycle test with an animal such as *Mysidopsis* or *Chironomus*. Additional possibilities include biochemical or other tests as indicators of potential reproductive, carcinogenic, or mutagenic effects. However, none of these tests has yet been developed, validated, or standardized sufficiently for routine regulatory application, and thus Tier IV evaluations are not recommended for implementation at the present time.

In summary, regulatory guidelines for evaluation and interpretation of petroleum hydrocarbons in dredged material are proposed as follows.

a. Determination of "reason to believe" that the sediment is contaminated with PAH (Tier I).

b. Analysis of dredged material for "total PAH" as the sum of the 15 key PAH and comparison with reference sediment (Tier II).

c. Biological testing of dredged material for acute toxicity and bioaccumulation, and comparison with test results from reference sediment (Tier III).

These three tiers are all currently feasible, and standard laboratory analyses are available. Recommendations that cannot be fully implemented immediately but that should be developed over the next few years include: compilation of a database relating environmental levels of the 15 PAH with biological effects, consideration of numeric regulatory criteria extracted from information in the database, and development of standardized sutlethal effects assessments for PAH (Tier IV).

PREFACE

Financial support for the second PAH workshop and preparation of this report was provided by the US Army Engineer District, Chicago, to the Environmental Laboratory (EL), US Army Engineer Waterways Experiment Station (WES), through an Intra-Army Order for Reimbursable Services.

The workshop proceedings were compiled by Ms. Joan U. Clarke, workshop chairperson, and Ms. A. Susan Jarvis, of the Ecosystem Research and Simulation Division (ERSD), EL. The compilers gratefully acknowledge the coordinating efforts of Mr. Jan Miller of the Chicago District and Ms. Carol Coch of the New York District. The compilers also thank all of the workshop participants and observers who provided detailed questionnaire responses and literature prior to the workshop, stimulating discussions and creative insights during the workshop, and helpful technical reviews of the draft Proceedings following the workshop.

This study was performed under the general supervision of Mr. Victor A. McFarland, Team Leader, Aquatic Contaminants Team; Dr. Lloyd H. Saunders, Chief, Contaminant Mobility and Regulatory Criteria Group; Mr. Donald L. Robey, Chief, ERSD; and Dr. John Harrison, Chief, EL.

COL Dwayne G. Lee, EN, was Commander and Director of WES at the time of the workshop. COL Larry B. Fulton, EN, is the current Commander and Director of WES. Dr. Robert W. Whalin is Technical Director.

This report should be cited as follows:

Clarke, Joan U., and Jarvis, A. Susan. 1990. "Regulatory Evaluation of Petroleum Hydrocarbons in Dredged Material; Proceedings of a Workshop," Miscellaneous Paper EL-90-11, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

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AGENDA

Tuesday, March 15, 1988 - Conference Room, Geotechnical Laboratory

8:30 a.m.	Welcome
8:45 a.m.	Opening remarks, COL Dwayne G. Lee, USA
9:00 a.m.	Background and objectives Chicago District New York District
10:00 a.m.	Break
10:15 a.m.	Review and discussion of 1986 workshop recommendations (Questionnaire Part A)
12:00 noon	Lunch
1:00 p.m.	Bulk chemistry as a tool/specification of threshold concentrations (Questionnaire Part B)
2:45 p.m.	Break
3:00 p.m.	Discussion of threshold concentrations and numeric guidelines (Questionnaire Part B)
4:30 p.m.	Return to hotel
6:30 p.m.	Dinner, Top O' The River

Wednesday, March 16, 1988 - Mississippi Room, Holiday Inn

8:30 a.m.	Discussion of biological effects of PAH (Questionnaire Part C)
10:00 a.m.	Break
10:15 a.m.	Discussion of biological effects of PAH (continued) (Questionnaire Part C)
12:00 noon	Adjourn until evening
6:30 p.m.	Discussion of sediment analyses and biological testing (Questionnaire Part B)
8:30 p.m.	Break
8:45 p.m.	Discussion of sediment analyses and biological testing (continued) (Questionnaire Part B)
10:00 p.m.	Adjourn

Thursday, March 17, 1988 - Conference Room, Geotechnical Laboratory

8:30 a.m.	Develop guidelines on biological testing and interpretation of results
10:00 a.m.	Break
10:15 a.m.	Develop guidelines on biological testing and interpretation of results (continued)
12:30 p.m.	Consensus luncheon (Monsour's); conclusion of workshop

ATTENDEES

Workshop Chairperson
Ms. Joan Clarke
U.S. Army Engineer Waterways Experiment Station, CEWES-ES-R
3909 Halls Ferry Road
Vicksburg, MS 39180-6199
601-634-2954

Technical Participants	Area of Expertise
Dr. Peter Landrum Great Lakes Environmental Research Laboratory 2205 Commonwealth Blvd. Ann Arbor, MI 48105-1593 313-668-2276	Aquatic toxicology, fate of organic pollutants, environmental chemistry, bioavailability
Dr. Richard Lee Skidaway Institute of Oceanography University System of Georgia P.O. Box 13687 Savannah, GA 31416 912-356-2494	Aquatic toxicology, bioaccumulation
Dr. Michael J. Mac US Department of the Interior Fish and Wildlife Service National Fisheries Center--Great Lakes 1451 Green Road Ann Arbor, MI 48105 313-994-3331	Aquatic toxicology, sediment bioassays, biological effects of chlorinated hydrocarbons, tissue residues in Great Lakes organisms
Dr. Joseph O'Connor Aquatic Habitat Institute 180 Richmond Field Station 1301 South 46th St. Richmond, CA 94804 415-231-9539	Toxicology, bioavailability, pharmacokinetics
Dr. Richard Peddicord EA Engineering Science & Technology 15 Loveton Circle Spartus, MD 21152 301-771-4950	Toxicity, bioaccumulation, ecological impacts of sediment-associated contaminants
Dr. Jim Petty U.S. Environmental Protection Agency EMSL-LV P.O. Box 93478 Las Vegas, NV 89193-3478 702-798-2381	Organic analytical chemistry, environmental contamination

Dr. Clifford Rice US Fish & Wildlife Service Patuxent Wildlife Research Center Laurel, MD 20708 301-498-0278	Analytical chemistry, transfer pathways in organisms, bioaccumulation in wildlife (birds), interface interactions, partitioning
Dr. Philippe Ross Illinois State Natural History Survey 607 E. Peabody Drive Champaign, IL 61820-6970 217-333-6897	Limnology, sediment bioassays, ecology of lake plankton, algae as bioindicators
Dr. Anne Spacie Department of Forestry and Natural Resources Forest Products Building Purdue University West Lafayette, IN 47907 317-494-3621	Marine/freshwater chemistry, analytical chemistry, bioassays, bioaccumulation in fish and crustaceans
Dr. Robert Spies, L-453 University of California Lawrence Livermore National Laboratory P.O. Box 5507 Livermore, CA 94550 415-422-5792	Benthic ecology, reproductive success and MFOs, fish physiology, petroleum seeps, sediment analysis, dredging
Dr. Dennis Stainken New Jersey Department of Environmental Protection Division of Environmental Quality Chief, Office of Quality Assurance CN 027 Trenton, NJ 08625 609-633-3840	Toxicology, chemistry, ecology, bioaccumulation, field research, effects on benthic populations
Dr. John Stein Environmental Conservation Division N.W. & Alaska Fisheries Center National Marine Fisheries Service/NOAA 2725 Montlake Blvd. E. Seattle, WA 98112 206-442-4638	Biochemistry, bioavailability, metabolism, effects on reproduction, fish tumors

WES PARTICIPANTS

Ms. Susan Jarvis, Technical Coordinator, CEWES-ES-R	601-634-2804
Dr. Tom Dillon, CEWES-ES-R	601-634-3922
Dr. Robert Engler, CEWES-EP-D	601-634-3624
Dr. Dick Lee, CEWES-ES-R	601-634-3585
Mr. Victor McFarland, CEWES-ES-R	601-634-3721
Dr. Henry Tatem, CEWES-ES-R	601-634-3695

U.S. Army Engineer Waterways Experiment Station
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

1988 PAH WORKSHOP CORPS DISTRICT SPONSORS AND OBSERVERS

Mr. Jan Miller[3]
U.S. Army Engineer District, Chicago
ATTN: CENCC-ED-HE
219 S. Dearborn St.
Chicago, IL 60604-1797
312-353-8576

Ms. Carol A. Coch[4]
U.S. Army Engineer District, New York
ATTN: CENAN-OP-W
26 Federal Plaza
New York, NY 10278-0090
212-264-5621

Dr. Tom Fredette
U.S. Army Engineer Division, New England
ATTN: CENED-OD-R
424 Trapelo Road
Waltham, MA 02254-9149
617-647-8057

Mr. John R. Adams
U.S. Army Engineer District, Buffalo
ATTN: CENCB-ED-HQ
1776 Niagara Street
Buffalo, NY 14207-3199
716-876-5454 (ext 2268)

Mr. Mike Lee
U.S. Army Engineer Division, Pacific Ocean
ATTN: CEPOD-CO-O
Bldg. 230
Fort Shafter, HI 96858-5440
808-438-9258

Mr. Rudd Turner
U.S. Army Engineer District, Portland
ATTN: CENPl-PL-A
P.O. Box 2946
Portland, OR 97208-2946
503-221-6401

---

3. Current address: U.S. Army Engineer Division, North Central, ATTN: CENCD-ED-WL, 536 S. Clark St., Chicago, IL 60605-1592; Phone 312-353-6354

4. Current address: U.S. Army Engineer Division, North Atlantic, ATTN: CENAD-PL-R, 90 Church St., New York, NY 10007-2979; Phone 212-264-7814

OTHER OBSERVERS

Ms. Jennifer Brown
Environmental Review Branch
5ME-14 USEPA
230 S. Dearborn St.
Chicago, IL 60604
312-886-6873

Mr. David Norris
Environmental Review Branch
5ME-14 USEPA
230 S. Dearborn St.
Chicago, IL 60604
312-886-6872

Dr. Mike Weinstein
Envirosphere Company
160 Chubb Ave.
Lyndhurst, NJ 07071-3586
201-460-6501

REGULATORY INTERPRETATION OF PETROLEUM HYDROCARBONS IN DREDGED MATERIAL

PROCEEDINGS OF A WORKSHOP

PART I: BACKGROUND

Summary of Scope of Work

This section summarizes the Scope of Work provided in Appendix A, and describes the background for both the first and the second PAH workshops.

In response to concerns over possible environmental impacts of dredging and dredged material disposal, regulatory analyses of dredged material and tissues of animals exposed to it have included quantitation of total oil and grease or total petroleum hydrocarbons. Over the last several years, scientific advances have made such limited quantitation of petroleum hydrocarbons inadequate for assessing the potential for environmental impact or for addressing concerns expressed by the public or by other agencies.

On the other hand, to comprehensively analyze for all possible petroleum hydrocarbons would be prohibitively time consuming and expensive. Hundreds of hydrocarbon compounds have been identified in sediment, water, and tissue samples. These compounds differ widely in water solubility, persistence, bioavailability, toxicity, and overall biological importance.

An intermediate approach is needed for an informed regulatory evaluation of the potential environmental impact of petroleum hydrocarbons in dredged material. The regulatory complexity of petroleum hydrocarbons needs to be simplified by focusing on clearly identified compounds or classes of compounds that have been shown to be of the most environmental importance. This would allow defensible evaluations at a time and cost that are reasonable in the Corps' dredged material regulatory program.

In May 1985 the WES received written requests from Mr. James Mansky of the USAED, New York, and Mr. Jan Miller of the USAED, Chicago, for assistance in the regulatory evaluation of petroleum hydrocarbons in dredged material. The WES provided this assistance in the form of two workshops on petroleum hydrocarbons in dredged material, the first one focusing on regulatory identification and the second one focusing on regulatory interpretation.

The First (1986) PAH Workshop

Introduction

The first PAH workshop was conducted 13-15 May 1986 at the WES. Ten technical participants were chosen from government agencies, academia, and private industry to represent a diversity of scientific backgrounds in environmental chemistry and the knowledge of biological effects of petroleum hydrocarbons in sediment. Prior to the workshop, the participants submitted their perception of selecting key compounds that would be of use in the regulatory evaluation of petroleum hydrocarbons in dredged material. During the workshop, the participants briefly introduced topics pertaining to their area of expertise. Topic introductions were followed by roundtable discussion.

The consensus recommendation of the participants was to use 15 specific priority pollutant PAH (Figure 1) in a suggested tiered testing approach for regulatory evaluation of hydrocarbon-contaminated dredged material. The 15 selected PAH were considered amenable to reliable chemical analysis by commercial laboratories, and to have a range of biological effects sufficiently representative of adverse biological impact due to petroleum and other hydrocarbon contamination in sediment.

The suggested tiered testing approach (Figure 2) is based on the assumption of a "reason to believe" that a sediment is contaminated with PAH. The first testing tier would consist of chemical analysis of the sediment for the 15 selected PAH, along with an acute toxicity test. If Tier I results were acceptable, then PAH need not be considered an issue of concern in the evaluated sediment. On the other hand, if Tier I showed unacceptable acute toxicity or indicated sediment PAH levels high enough to be of concern, then Tier II would be conducted. Tier II testing would consist of bioaccumulation tests to determine if the PAH are bioavailable. The bioaccumulation tests would employ organisms such as bivalve molluscs that have limited ability to metabolize PAH and thus are capable of bioaccumulating the parent compounds. Results of the tiered testing approach would be used in the regulatory decision-making process for dredging and disposal options.

Several additional classes of hydrocarbons were recommended for further study because of their potential environmental and toxicological significance. However, these studies are secondary to research needs on the 15 PAH, their metabolism and sublethal effects, in the ultimate development of sound, feasible regulatory guidance concerning hydrocarbon-contaminated sediment.

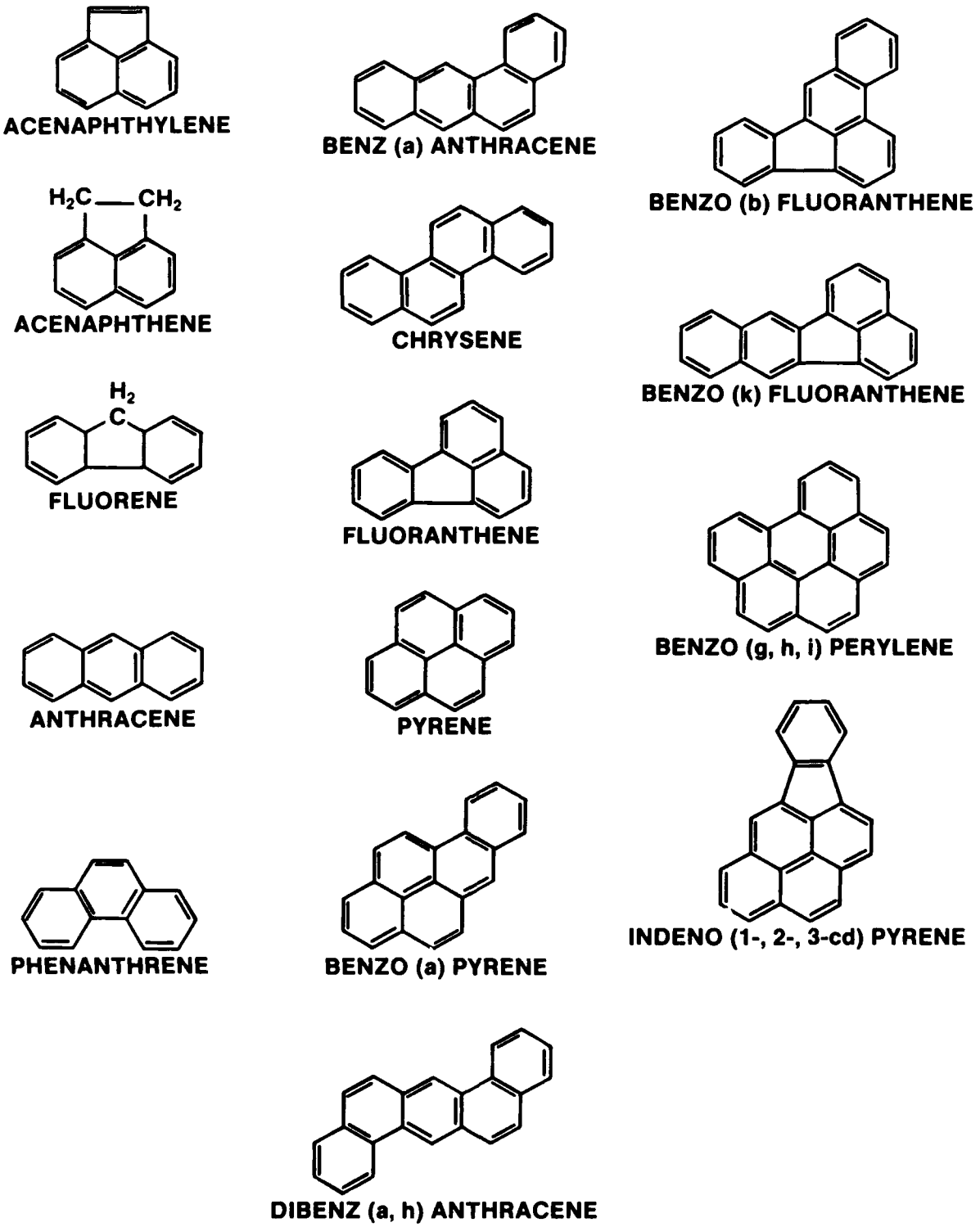

Figure 1. Priority pollutant polycyclic aromatic hydrocarbons recommended by the 1986 and 1988 PAH workshops for use in regulatory evaluation of dredged material

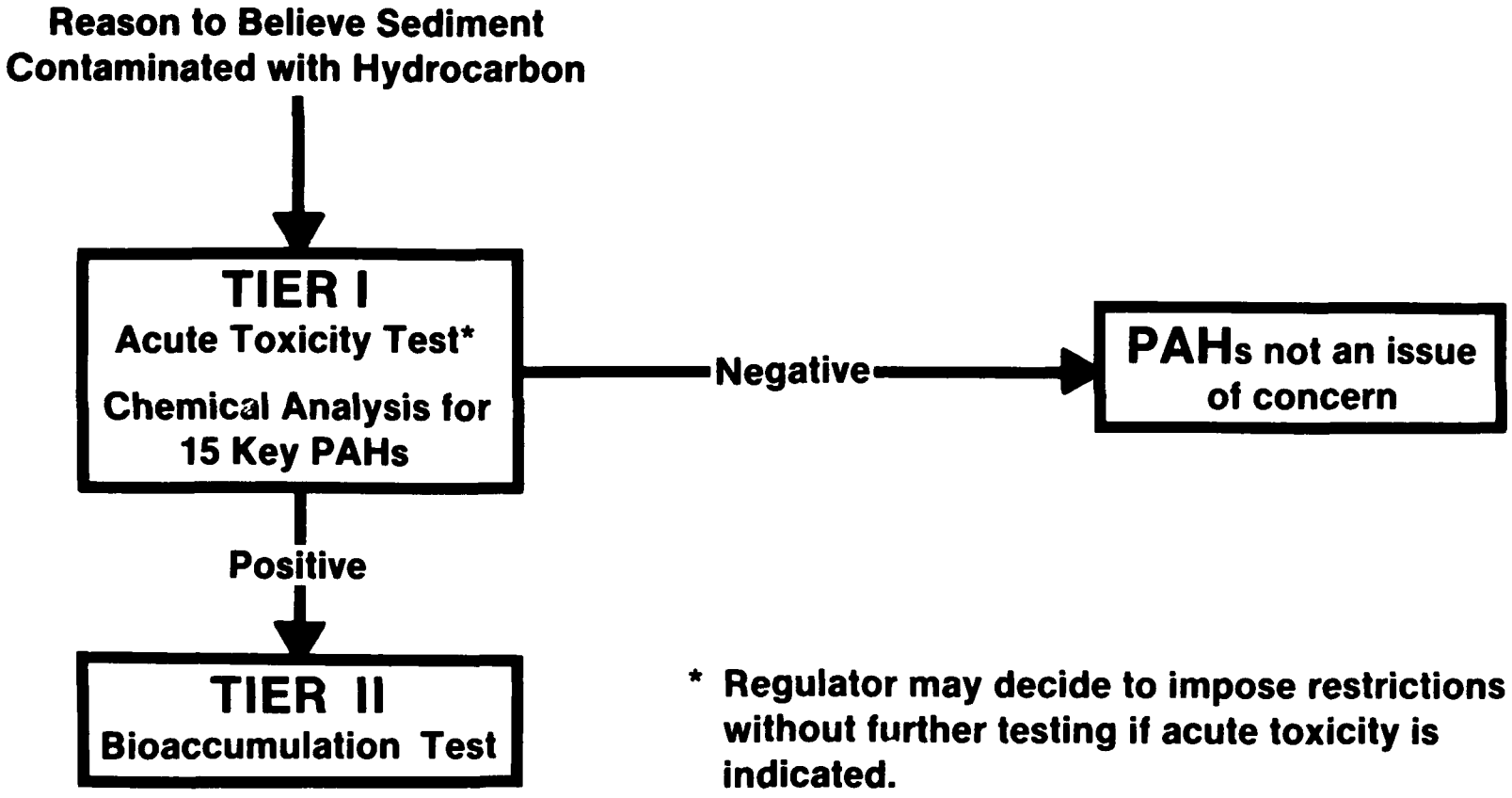

Figure 2. Tiered testing approach recommended by the 1986 PAH workshop for regulatory evaluation of dredged material

Proceedings of the 1986 PAH workshop are summarized in Clarke and Gibson (1987a,b) and Clarke (1987).

Summary of major agreements

The major agreements reached by the 1986 PAH workshop participants may be summarized as follows (Clarke and Gibson 1987a, Part III):

a. The oil and grease test does not provide a meaningful summary measure of hydrocarbon contamination in sediment. At the other extreme, analyses for all petroleum hydrocarbons as individual compounds would be too difficult, costly, and uninterpretable. An intermediate approach is needed for regulatory evaluation.

b. "Petroleum" is too restrictive a term, and any hydrocarbon contamination of dredged material should be considered, regardless of source of the hydrocarbons.

c. Aliphatic hydrocarbons need not be included in regulatory evaluations because they may pose analytical difficulties and generally do not cause major environmental impacts in the context of dredging and disposal.

d. Polycyclic aromatic hydrocarbons (PAH) are the most important class of hydrocarbon contaminants in dredged material due to their toxicity and persistence.

e. Analysis for a limited number of specific PAH would have better interpretability than analyses for ring classes or groups based on log P ranges.

f. The list of compounds recommended for regulatory evaluation of hydrocarbons in dredged material includes the following 15 priority pollutant PAH:

(1) Acenaphthene
(2) Acenaphthylene
(3) Anthracene
(4) Benz[a]anthracene
(5) Benzo[a]pyrene
(6) Benzo[b]fluoranthene
(7) Benzo[g,h,i]perylene
(8) Benzo[k]fluoranthene
(9) Chrysene
(10) Dibenz[a,h]anthracene
(11) Fluoranthene
(12) Fluorene
(13) Indeno[1,2,3-cd]pyrene
(14) Phenanthrene
(15) Pyrene.

Naphthalene, which is also considered a priority pollutant PAH, has not been included in this list because it is too volatile to give accurate analytical results and too water soluble to persist in

sediments. It was felt that a high level of naphthalene would be manifested as mortality in acute toxicity tests.

g. A tiered testing approach to regulatory evaluations of PAH in dredged material was recommended. This would begin with a general assessment of the likelihood of contamination. The first testing tier would include an acute toxicity test and analysis of the sediment for the 15 priority pollutant PAH. The second-tier test would consist of a 10-day bioaccumulation test to demonstrate bioavailability.

h. In assessing the potential for bioaccumulation, organisms that have limited or no ability to metabolize PAH should be used. Analysis of tissues for unmetabolized parent compounds is thus simplified. The group suggested the clam *Mercenaria* or a suitable substitute bivalve, or an amphipod such as *Pontoporeia*, as appropriate species to use in the 10-day bioaccumulation test.

i. The group recommended against analysis for metabolites of PAH in a routine regulatory program until more research is completed and analytical methods are better established.

j. A critical need is QA/QC evaluations and procedures, especially when a variety of laboratories are used by a regulatory agency for testing and review purposes.

k. Recommendations for future research focus on the development of analytical procedures and biological testing protocol for the evaluation of alkylated PAH, and of representative hydrocarbons and derivatives from classes other than the PAH. These include the nitrogen-, sulfur-, and oxygen-containing heterocycles (particularly the acridines and thiophenes), nitroaromatics, and aromatic amines.

l. Biological tests that need to be refined and standardized include assays for carcinogenicity, genotoxicity, reproductive effects, and photoinduced toxicity.

The Second (1988) PAH Workshop

Regulatory perspectives

The two USAE Districts sponsoring the workshops have quite different problems and approaches concerning the regulation of dredging and disposal operations. The waters encompassed by the Chicago District are entirely fresh, primarily large lake systems and their tributaries. Regulatory agencies around the Great Lakes have traditionally accepted bulk sediment chemistry as the means of evaluating disposal alternatives for contaminated dredged material. Disposal guidelines are based on the similarity of chemical and physical characteristics between the dredged material and disposal site sediment. The New York District, on the other hand, includes primarily coastal

marine and estuarine environments. Testing criteria for ocean disposal require biological testing.

The USEPA Region V has used a rigid classification scheme, based on bulk chemistry, to characterize sediment as "nonpolluted," "moderately polluted," "heavily polluted," etc. Disposal options are determined from this classification. This regulatory system could potentially be applied, with little scientific backing, to the evaluation of PAH-contaminated sediment. Thus the Chicago District is interested in developing a scientifically credible approach to regulation of PAH-contaminated dredged material, before the traditional classification scheme becomes entrenched for PAH.

Sediment may be euphemistically characterized as "white," "black," or "gray," referring, respectively, to uncontaminated sediment suitable for open water disposal, to highly contaminated sediment that would not be considered for unrestricted open water disposal, and to sediment with contaminant levels falling in between the black and the white levels. Some extremes in the Great Lakes can already be identified. Sediment in some parts of Indiana Harbor, for example, is decidedly "black," whereas much of the surficial, silty sand, open lake sediment is "white." "Gray" sediment is typically found at the transition zone between urban harbors and the open lake. One utility of bulk sediment chemistry may be to distinguish those sediments that are "black" or "white" and would require no further testing. Comparison with ambient sediment contaminant levels has historically been the means for determining what is black and what is white in the Great Lakes.

The regulatory approach used by the New York District is based to a large extent upon ambient tissue levels of certain contaminants in specific species living in the apex of the New York Bight (but not including the Mud Dump Site where dredged material is disposed). The intent of this approach (termed the "matrix" approach by the New York District) is to prevent further degradation by prohibiting unrestricted ocean disposal of contaminated dredged material that has been shown to produce tissue levels higher than the ambient values.

Ambient tissue levels were determined for polychlorinated biphenyls (PCB), mercury, and cadmium, but none are available for PAH. Thus, the New York District has regulated dredged material containing PAH by comparing biological test (acute toxicity and bioaccumulation) results for the dredged material to those of an appropriate reference sediment but not to ambient values. If the biological test results for the dredged material are statis-

tically significantly greater than those for the reference, and the dredged material produces mortality at least 10 percent greater than the reference, then unrestricted ocean disposal is not permitted.

Under current dredging programs, about 6 percent of coastal dredging, nationwide, is judged unsuitable for unrestricted ocean disposal, and about 10 percent in the Northeast (US Congress, Office of Technology Assessment, 1987). In the New York area, about 5 percent of dredged material does not meet the Ocean Dumping criteria, and roughly an additional 5 percent can be ocean disposed but requires capping. The remaining 90 percent can be ocean disposed without restrictions. Other disposal options are severely limited due to the volume of dredged material and the lack of upland and wetland disposal sites near New York City. In the Great Lakes, on the other hand, under the chemistry-based regulatory approach, half of the dredging is considered unsuitable for unrestricted aquatic disposal.

The actual regulations governing disposal in inland and coastal waters under Section 404(b)(1) of the Clean Water Act, and those governing ocean disposal under Section 103 of the Ocean Dumping Act, do not differ greatly in their requirements. Both evaluate disposal using a tiered testing scheme that includes determination of "reason to believe" that the sediment is contaminated (Tier I), sediment chemistry inventory and physical measurements (Tier II), and biological assessment (Tiers III and IV). They differ mainly in that the Implementation Manual for Section 103 (EPA/CE 1977) specifies testing procedures in more detail than the Manual for Section 404(b)(1), and is oriented toward marine, rather than freshwater systems. To provide a nationally unified regulatory approach for both 103 and 404 evaluations, the Corps has developed a comprehensive testing strategy for aquatic disposal as part of the Federal Standard (33 CFR Parts 209, 335, 336, 337, and 338, Discharge of Dredged Material Into Waters of the U.S. or Ocean Waters; Operation and Maintenance; Final Rule; Federal Register, April 26, 1988). This comprehensive tiered testing approach is described by Engler et al. (1988).

Objectives

This section summarizes the objectives of the second PAH workshop, as stated by the District sponsors at the beginning of the workshop, and as listed in the Statements of Objectives provided by the Districts several months prior to the workshop. The Statements of Objectives are included as Appendix B to this report.

The primary objective of the second PAH workshop was to develop guidance on scientific interpretation of potential impacts of the 15 priority pollutant PAH selected at the 1986 PAH workshop. The Chicago and New York Districts wished to obtain recommendations for specific biological tests and organisms to be used in the tiered testing scheme for regulatory evaluation of PAH in dredged material. Both Districts also desired available information on range of concentrations of the 15 selected PAH, individually and collectively, that would cause effects in organisms.

An important objective noted by the Chicago District was to examine the possibility of establishing regulatory screening guidelines, such as upper and lower threshold concentrations, for PAH in sediment. The lower threshold concentrations would represent no significant contamination. Dredged material with PAH levels falling below those values ("white" sediment) could be approved for unrestricted open water disposal. The upper threshold concentrations, on the other hand, would represent "gross" levels of contamination. Dredged material with PAH levels above those values ("black" sediment) would not be considered for unrestricted open water disposal. Only sediment having PAH contaminant levels between the upper and lower threshold values (i.e., "gray" sediment) would require further testing before regulatory decisions concerning disposal could be made. For the "gray" sediment, the Chicago District sought recommendations on appropriate biological testing procedures.

The New York District expressed an interest in developing procedures to determine the environmental importance and extent of bioavailability of PAH in sediment. An important objective was to discuss what tissue concentrations of PAH could be considered biologically meaningful and how the uptake and effects of PAH might vary depending on the test organism selected. Another objective of the New York District was to determine whether the matrix approach could be implemented for PAH-contaminated sediment.

To best accomplish the objectives, 12 scientists from federal and state agencies, academia, and private industry were invited to participate in the second workshop. Participants were selected on the basis of their expertise in environmental chemistry, biological effects of PAH, and dredging and dredged material regulatory processes. Several scientists from the WES were also asked to participate. A representative was included from each of the USAED, Chicago and New York. Scientists and regulators from other Corps Districts and Divisions, the USEPA, and private industry who wished to attend the workshop were welcomed as observers. Roundtable discussions during the work-

shop centered on the workshop objectives, questionnaire responses, and other issues raised by the District sponsors. After the workshop, this report was prepared summarizing the workshop goals, activities, conclusions, and recommendations.

Preworkshop questionnaire

Prior to the workshop, invited participants were asked to submit written answers to specific questions provided to them in the form of a questionnaire. Ten participants submitted responses to the questionnaire, which were compiled and are provided as Appendix C to this report. Summaries of the responses are also integrated into Parts II and III of this report.

The questionnaire was divided into three sections. The first section dealt with a re-examination of the recommendations of the 1986 PAH workshop. The second group of questions covered sediment analyses and biological testing while the third section was concerned with the biological effects of PAH. The questions in the preworkshop questionnaire were drawn from issues raised at the end of the 1986 PAH workshop and also by the reviewers of the first PAH workshop Proceedings (Clarke and Gibson 1987a).

PART II: PROCEEDINGS OF THE WORKSHOP

Introduction

Following the opening remarks by COL Dwayne G. Lee, Ms. Joan Clarke, the workshop chairperson, welcomed the participants and asked them to introduce themselves. She then emphasized the primary objective of the workshop, the regulatory interpretation of PAH in dredged material. Ms. Clarke requested the workshop participants to build on the 1986 workshop recommendations by specifying appropriate biological tests and organisms for use in the tiered testing approach, and to assess, insofar as possible, the ecological significance of the 15 PAH in dredged material. As a starting point, it was considered desirable to briefly re-evaluate the recommendations of the 1986 PAH workshop in light of research during the two years between the workshops. New findings might necessitate changes in the list of key compounds or tiered testing approach proposed at the 1986 workshop.

Reevaluation of PAH Selected by the 1986 Workshop

Part A of the preworkshop questionnaire (Appendix C) posed three questions relating to the 1986 workshop selection of 15 PAH (Figure 1) for regulatory evaluation of petroleum hydrocarbon-contaminated dredged material:

A.1. Do you feel strongly that any compounds should be added to or deleted from the list? If so, please state your justifications.

A.2. Are there any groups of compounds that have shown parallel changes in concentration, possibly indicating a common source and the need to analyze for only one of them?

A.3. Can any of the PAH be used as target compounds for specific environments or incidents (e.g. runoff, leaching, creosote, oil spill)? What is the most likely source for each of the 15 PAH in the aquatic environment, particularly New York Harbor and the nearshore areas of the Great Lakes?

Pertaining to Question A.1, six respondents said that no changes should currently be made to the list of 15 PAH. Others suggested several compounds for addition to the list, including naphthalene, 1-methylnaphthalene, dimethylnaphthalene, benzo[e]pyrene, and dibenzothiophene. Some respondents noted

that certain classes of hydrocarbons, such as the azaarenes, aromatic amines, sulfur-containing aromatics, and alkyl-substituted PAH, may be important toxicologically. However, knowledge of these compounds and their effects is limited at present.

Dr. Dennis Stainken presented a strong case for the inclusion of one or more of the naphthalenic PAH in the list. He indicated that naphthalene, dimethylnaphthalene, and other methyl-substituted naphthalenes are prevalent in the New York Harbor, and are relatively stable at the ambient sediment temperatures. These compounds are important in that they can cause immediate, acutely toxic effects. Dr. Henry Tatem noted that naphthalene constituted the major portion of total quantified PAH in Indiana Harbor sediment (Environmental Laboratory 1987), and that bioaccumulation studies from other locations have demonstrated the importance of dimethylnaphthalene (Neff 1979). Dr. Stainken maintained that commercial laboratories should be able to analyze naphthalene without difficulty, and that volatilization of naphthalene from sediment should not be a problem at the low temperatures typical of New York Harbor.

Several participants, however, raised compelling objections to including naphthalene on the list. Dr. Jim Petty agreed that naphthalene would not volatilize substantially from sediment at low temperatures, but noted that sublimation of naphthalene during sample preparation would be more of a problem than volatilization. He maintained that commercial laboratories would have difficulties with both accuracy and precision in analyzing naphthalene. Mr. John Adams added that naphthalene in high concentrations can be analytically problematic. Dr. Peter Landrum recalled the 1986 workshop consensus that naphthalene is biologically important in terms of acute but not chronic toxicity, and that acute toxicity attributable to naphthalene should be adequately detected in the Tier I toxicity test.

Some discussion ensued as to the utility and necessity of doing chemical analyses on sediment. Dr. Tom Fredette pointed out that biological testing may not be economically viable for small dredging operations, and that bulk sediment analysis gives these applicants a screening tool for identifying sediments that are low in contaminants and would not need biological testing. Dr. Petty concurred that the cost of chemical analysis is less than that of biological testing, and added that chemical analysis provides information on which to judge bioaccumulation potential of contaminants. Dr. Michael Mac stated that bulk chemical analysis will help to define areas of contamination

in heterogeneous mixtures of sediment, and perhaps more importantly, will assist in explaining bioassessment, particularly bioaccumulation, results. Sediment bulk chemistry can provide qualitative guidelines concerning what compounds to expect or request in the tissue analyses of the bioaccumulation tests.

Mr. Adams noted that the list of 15 PAH does not include any alkyl-substituted PAH, and asked whether the 15 selected PAH are sufficient surrogates for toxicity range and mechanisms of effect to represent the entire class of PAH. Should an alkyl-substituted PAH, such as 1-methylnaphthalene or dimethylnaphthalene, be added because this group of compounds is different and behaves differently from the 15? In response, Dr. Richard Peddicord referred to the 1986 workshop recommendation that further research be conducted on representative members of several classes of hydrocarbons or petroleum derivatives, including the alkyl-substituted PAH. He noted that the 15 PAH were chosen, in part, because they are priority pollutants, and thus there is widespread recognition of their importance, not necessarily that they are surrogates for all possible mechanisms and effects of all petroleum hydrocarbons.

Dr. Landrum stated that benzo[e]pyrene should not be added to the list because it does not create chronic toxicity, and is quite similar to benzo[a]pyrene in terms of partitioning and bioaccumulation. Dr. Mac noted that benzo[e]pyrene is a suspected carcinogen and occurs in a number of Great Lakes harbors in almost equal concentrations to benzo[a]pyrene. However, Dr. Landrum indicated that benzo[e]pyrene is a very weak carcinogen compared to benzo[a]pyrene (Lehr et al. 1980), and that its inclusion on the list would not greatly enhance our assessment of the hazard associated with contaminated sediment. Other workshop participants readily agreed that there was no need to add benzo[e]pyrene to the list at this time.

Although the participants were not in complete agreement that no compounds should be added to the list, there was general consensus that the list of 15 PAH should remain unchanged for the time being. This agreement was influenced by several factors:

a. As the chemical analysis becomes more complicated by the addition of compounds, the cost goes up and the level of confidence in and interpretability of the data produced go down.

b. Standard analytical protocols could misidentify most of the additional compounds under consideration, and special procedures would have to be used.

c. Chemical analysis of the sediment alone is not sufficient to identify potential toxicity problems because toxicity may be caused by compounds present in the sediment but not included in the chemical analysis. Acute toxicity tests are necessary in addition to the sediment analysis, as recommended by the 1986 PAH workshop for Tier I testing.

d. Acute toxicity tests can demonstrate adverse biological effects due to the more volatile hydrocarbons such as naphthalene, xylene, and benzene.

e. In the event that acute toxicity tests are not done, it would be necessary to analyze sediment for some of the more volatile hydrocarbons in addition to the 15 PAH.

Concerning Question A.2, the consensus of the questionnaire respondents and of the workshop participants in general was that there are no groups of PAH showing parallel changes in concentration in all situations. The 15 selected PAH behave differently from each other and may derive from different sources. Thus, all of them should be analyzed. However, Dr. Robert Spies stated in his response that concentrations of 13 PAH analyzed in about 40 San Francisco Bay sediment samples were all highly correlated with each other. He suggested that although measurement of a few of these compounds could enable reasonable predictions of the others, this approach would not likely result in substantial cost savings.

Because the 15 selected PAH derive from many different sources, it would be difficult to use them for source studies. This was the consensus response to the first part of Question A.3. A major source of PAH, especially to the Great Lakes, is atmospheric deposition, primarily of the high molecular weight PAH. Other sources include crude or refined oil (mainly low molecular weight PAH); combustion by-products (both high and low molecular weight PAH), with low-temperature combustion leading to alkylated compounds and high-temperature combustion leading to unalkylated compounds; coking and aluminum smelting (mainly around the Great Lakes); urban runoff and waste material discharge (of considerable importance in the New York Harbor); and creosote operations. Dr. Stainken mentioned that naphthalenes in the environment are an indicator of fresh petroleum.

Summarizing the reevaluation of the 1986 workshop recommendations, most participants agreed that:

a. No new compounds should be added to the list of 15 PAH recommended for regulatory evaluations at the present time.

b. None of the 15 PAH should be deleted from the list.

c. Classes of hydrocarbons other than the priority pollutant PAH, such as the alkyl-, nitrogen-, and sulfur-substituted PAH, could have major toxicological importance and should be studied further.

Agreement on point a was not unanimous. Although analytical chemists in the group highlighted difficulties with the analysis of naphthalene, at least two participants felt strongly that naphthalene should nevertheless be included in the list of selected PAH.

The tiered testing scheme proposed by the 1986 workshop will be discussed later in this report.

Bioavailability of PAH

Consideration of the adverse biological effects of sediment-associated PAH on organisms is relevant only to the extent that PAH are available to the organisms. The question of PAH bioavailability from sediment was not discussed to any great length during the workshop, but Dr. Richard Lee noted in his questionnaire response that bioavailability of PAH from sediment has been demonstrated by a number of investigators (Landrum et al. 1985, McCain et al. 1978, Tatem 1986, Varanasi et al. 1985).

Dr. Stainken pointed out that the extent of bioavailability is influenced by complex environmental interactions. In the lower New York Harbor area, PAH bioavailability changes with seasonal cycling and with distance above the bottom. He indicated that PAH occur in the sediments and perhaps in the surface water microlayer, but in numerous surveys of the area he found it difficult to measure any PAH 1 m above the bottom or anywhere up in the water column. He suggested that in a regulatory program, one of the first evaluations should be a measure of immediate bioavailability, i.e., potential for bioaccumulation.

Biological Effects of PAH

Ms. Clarke asked the participants to turn their attention to the biological effects of PAHs and outlined several questions to keep in mind during the ensuing discussions:

a. What biological effects can be specified?

b. What residue/effects information is available?

c. How do species differ from one another in effects and sensitivities?

These questions relate directly to the two questions in Part C of the pre-workshop questionnaire (Appendix C):

C.13. Please list any information you have concerning specific levels of the 15 PAH that can be related to specific adverse biological effects in specific organisms.

C.14. Briefly and in general, how do the biological effects of PAH differ, qualitatively and quantitatively, among different groups of organisms? Do the species [recommended in response to Question B.9] differ from each other in sensitivity to PAH?

In addition, the response to Question b above will pertain to two additional questions posed in Part B of the questionnaire:

B.4. Can you specify for any of the 15 PAH a "level of concern" [lower threshold] in sediments or in tissues, below which that compound is unlikely to have any adverse biological effects?

B.5. Can you specify for any of the 15 PAH a threshold level of "gross" contamination [upper threshold] in sediments or in tissues, above which that compound will most likely have unacceptable adverse biological effects?

In other words, can residue/effects data be used to derive thresholds to indicate the "gray"-to-"black" cutoff (upper threshold) and the "white"-to-"gray" cutoff (lower threshold) for sediment (or for tissues)? Another question from Part B of the questionnaire relates to the topic of criteria:

B.6. Do any type of defined regulatory criteria exist for any of the 15 PAH?

Types of biological effects

Acute toxicity. PAH levels in many sediments are not likely to be acutely toxic, although Dr. Landrum suggested that a highly contaminated sediment having total PAH in the parts-per-thousand range could well be associated with acute toxicity to some organisms. He added that in his investigations, mixtures appear to be a little more toxic than single PAH compounds. He found phenanthrene to be toxic at 120 parts per million (ppm) in sediment with 1 percent total organic carbon (TOC). In relatively large concentrations, many of the PAH could be acutely toxic.

Dr. Landrum described increasing acute toxicity with increasing number of rings in the PAH molecule. Aqueous solubility, however, tends to decline with increasing molecular weight. Thus, as the PAH increase in size, the toxic concentration eventually exceeds the aqueous solubility, and not enough of the compound can be present in water to cause acute toxicity. Dr. Landrum suggested that in sediment, acute toxicity may be related to factors other than molecular weight and aqueous solubility of the PAH. Dr. Tom Dillon noted that biological effects, whether acute or chronic, cannot be inferred directly from PAH concentrations in sediment because of the complexity of various contaminant interactions, along with other factors such as ammonia, sulfides, dissolved oxygen, grain size, etc.

Dr. R. Lee proposed that the toxicity of PAH in sediment will be due primarily to that fraction of the PAH entering either the water column or the sediment intersitial (pore) water. Dr. Landrum noted that desorption is necessary, in that the PAH molecule must leave the sediment particle to which it is sorbed and pass through some aqueous phase, be it at the gill membrane or in the gut, to enter the tissue. This process will be inversely proportional to the degree of binding to the sediment particle. He indicated that ingestion of sediment can be a very important factor in toxicity or at least in bioaccumulation. In the Great Lakes amphipod *Pontoporeia hoyi*,[5] for example, sediment ingestion may account for up to 100 percent of PAH bioaccumulation (Landrum, in review). For the more water soluble compounds, desorption and uptake from interstitial water seems to be the dominant route.

Reproduction and growth. Dr. R. Lee emphasized adverse impacts on reproduction and growth as the effects that will have the most ecological importance to a population over the long term. He indicated that effects of benzo[a]pyrene on steroid metabolism in mammals have been documented, and that benzo[k]fluoranthene and benzo[b]fluoranthene may have effects similar to benzo[a]pyrene. However, he also stressed that effects of most of the 15 selected PAH on reproduction and growth have not been well studied, and in general there is not yet sufficient information to say that one compound is more important than another in this regard.

Dr. R. Lee mentioned a study of the polychaete worm *Nereis virens* in heavily oil-contaminated areas near Portland, Maine (Fries and Lee 1984). The

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5. Species mentioned in this report are listed in Table 1, along with bioassessment tests and test media for which their use was recommended, and any comments made about the species during the workshop.

investigators found defined year classes of the worms from larvae that settled in the area periodically, but no evidence of reproduction in this population. During the three years of the study, no gametes were seen in mature worms. Dr. Lee believed that the lack of reproduction could be attributed to the high levels of petroleum hydrocarbons. Dr. Landrum also related petroleum hydrocarbon exposure to developmental abnormalities in marine organisms such as sea urchin larvae.

Carcinogenicity/mutagenicity. Dr. Landrum indicated that the PAH having structural bay regions (see Clarke and Gibson 1986a, Figure 2) may be suspected as carcinogens. Among the 15, benz[a]anthracene, chrysene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[b]fluoranthene, and benzo[k]fluoranthene have demonstrated carcinogenicity in mammalian systems. Dr. Landrum knew of only two laboratory studies using fish, in which benzo[a]-pyrene and benz[a]anthracene were shown to produce carcinomas, a type of malignant tumor (Schultz and Schultz 1982, Hendricks et al. 1982). However, Dr. Petty suggested that PAH known to be mammalian carcinogens have a high potential to cause cancer in other biological systems, because the mechanisms of carcinogenicity are the same. Dr. Landrum agreed that the metabolic biotransformation routes in fish are very similar to those that have been linked with carcinogenic effects in mammals.

Ms. Carol Coch inquired whether similar biotransformation mechanisms and carcinogenic effects would occur in benthic invertebrates as in fish. None of the participants mentioned having observed tumors in benthic invertebrates. Biotransformation, however, does occur in some benthic organisms but apparently not in others. Dr. Landrum noted that chironomids (midges) exhibit the same type of biotransformation mechanisms as fish. In *Pontoporeia*, however, he was not able to measure biotransformation. In the mysid shrimp *Mysis relicta* there is a slight amount of biotransformation. Dr. R. Lee stated that some marine crustaceans biotransform very rapidly; however, biotransformation ability in bivalves is limited. Dr. John Stein also noted that there was moderate biotransformation in the west coast marine amphipod *Rhepoxynius abronius*, but very little in another amphipod, *Eohaustorius washingtonianus* (Reichert, Eberhart and Varanasi 1985). Dr. R. Lee suggested that there is usually an inverse relationship between bioaccumulation and biotransformation.

Mr. Jan Miller asked whether an organism's inability to biotransform indicates that chronic effects will be limited or nonexistent. Several

participants said not necessarily, although Dr. R. Lee suspected that most biological effects of PAH other than acute toxicity are due to biotransformation products. In other words, the compounds that are active carcinogens or mutagens also tend to be active in terms of producing other responses, such as effects on growth and reproduction. Thus, he proposed that organisms like molluscs having limited ability to biotransform PAH are unlikely to experience carcinogenic effects or impairment of growth and reproduction due to PAH exposure. Dr. Dillon noted, however, that Widdows, Donkin and Evans (1987) demonstrated an inverse relationship between a particular measure of growth ("scope for growth") in the mussel *Mytilus edulis* and tissue concentration of aromatic hydrocarbons. Some populations of molluscs have relatively high incidences of tumors, but extensive field studies reviewed by Mix (1988) have failed to implicate PAH contamination as the causative agent, and suggest that viruses or some other pathogen may be responsible.

Tumors in fishes, on the other hand, show a more definitive link with environmental contamination (Malins et al. 1988, Couch and Harshbarger 1985). However, cancers in fishes are not necessarily an indication of detrimental, long-term, ecological effects, according to Dr. Joe O'Connor. He described tomcod (*Microgadus tomcod*) in the Hudson River, New York, as having 85-100 percent incidence of cancer in the population over 1-1/2 to 2 years of age. Yet these individuals are surviving, growing, and reproducing. The age structure of the population has changed since the early part of the century but the species remains an ecological dominant despite the high incidence of cancer. Furthermore, species diversity of finfish has increased in the Hudson. Thus, long-term environmental significance cannot be ascertained merely from the presence of cancers. Dr. Mac argued that species like walleye or striped bass, which are subject to more predation pressure than the tomcod, could very well be adversely affected ecologically by a high incidence of cancer that kills off the older fish and prevents individuals from reproducing for more than one or two years. Dr. O'Connor replied that through ecological time species come and go, and that a species adversely affected by some carcinogenic compound will be replaced by another species that is more tolerant of the effects of that compound. Therefore, on an environmental or ecological scale, changes in a single population may not be so important. Dr. R. Lee agreed, and noted that fish can have an induced mixed-function oxidase (MFO)

system[6] in areas of high oil contamination, and yet grow and reproduce normally (Kezic et al. 1983, Payne and May 1979, Payne and Penrose 1975, Payne et al. 1984).

Dr. Michael Weinstein commented that population problems such as cancer are not likely to be caused by PAH in isolation, but by other environmental stresses as well. PAH, particularly in industrialized areas, will not be the sole source of stress to a population but will act in concert with metals, other organics, low dissolved oxygen, and other factors. Dr. Dillon noted the evidence that at least some cancers in aquatic organisms are caused by viruses (Mix 1986).

Effects-based screening guidelines

Dr. R. Lee summarized the response to Question C.13 by stating that there is currently insufficient information concerning specific levels of any of the 15 selected PAH in sediment that cause adverse biological effects in specific organisms, on which to base "levels of concern" or any kind of screening guidelines. Dr. Stainken, however, felt that the information sources from which the participants drew in composing their responses to Question C.13 represent only a portion of what is available in the literature. A thorough search of the literature might yield a sufficient body of data to assist in the development of guidelines. Such a search would require a massive effort, which was clearly far beyond the scope of the questionnaire response and workshop preparation.

Dr. Peddicord suggested using a partitioning approach on water concentrations of PAH known to cause adverse effects, to arrive at a rough indication of effects concentrations for PAH in sediment. Dr. Stein said that this approach has been tried, and that understanding of the processes involved is in general too limited to allow derivation of useful approximations for sediment. Dr. Landrum observed that there are also problems with selecting or measuring appropriate partition coefficients to use in the approach. Dr. Petty added that calculation of effects concentrations for sediment would have to take into account both the amount and the kind of organic carbon in the

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6. The MFO system functions in the biotransformation of xenobiotic compounds such as PAH [see, for example, Buhler and Williams (1988) for a description of xenobiotic biotransformation processes], and in the metabolism of reproductive steroids. Exposure to certain xenobiotics, such as PAH and some of the PCB congeners, results in induction or "turning on" of the MFO system.

sediment. Values would have to be derived for a range of organic carbon contents. He and Dr. Landrum agreed that because of the complexity of the physical, chemical and biological factors involved in this approach, the calculations would generate orders of magnitude of uncertainty. Dr. Mac suggested that developing sediment-based bioassays for use in regulatory decision making concerning sediment is more feasible than trying to derive screening guidelines for sediment from the results of bioassays using PAH in water.

Other experimental approaches that could potentially be applied to the development of thresholds or screening guidelines for PAH in sediment were discussed during the workshop. These include the screening level concentration (SLC) approach (Neff et al. 1987), and the sediment quality triad (Chapman 1986; Chapman, Dexter and Long 1987). There was also some discussion of the Chapman et al. (1987) comparison of these two approaches with two others (apparent effects threshold (Barrick et al. in preparation), and laboratory sediment bioassays using *Rhepoxynius abronius* (Swartz et al. 1985)). However, use of these approaches by Corps Districts for regulation of dredged material is not advocated for two reasons. First, these approaches are still developmental and have unresolved technical deficiencies. They have not yet been field verified to determine their general applicability in any portion of the regulatory program. Site specificity and other technical weaknesses of the approaches were pointed out by several workshop participants. Second, it is unclear at present how these approaches would fit into the Corps regulatory program. Although the Clean Water Act allows chemistry-based regulatory decisions (even though the preferred approach is effects-based), the approaches mentioned here were developed in saltwater and none of them has been evaluated in the freshwater environment.

Total versus individual PAH. Dr. Philippe Ross suggested that there may be more promise in determining screening guidelines for total PAH than for each of the 15 selected PAH individually. He noted the importance of generating guidelines realistically using real sediment containing PAH in the presence of other pollutants. Dr. Dillon agreed that given the paucity of data on individual PAH, focusing on total PAH may be more appropriate and realistic in responding to the Districts' needs. "Total PAH," however, can be measured in many different ways. For Corps regulatory purposes, it would make most sense to "standardize" total PAH as the sum of the 15 selected PAH. Even so, Dr. Peddicord pointed out that total PAH would refer to a mixture likely to have a different relative composition of the 15 compounds in every sedi-

ment. He emphasized the need to develop a database relating biological effects to sediment levels for each of the individual 15 PAH. He suggested that sediment approved for open water disposal by currently applied criteria be analyzed for the 15 PAH to determine what might be considered "acceptable quality" for sediment on the basis of the 15 PAH. However, Mr. Miller pointed out that the Corps in a regulatory capacity cannot ask a permit applicant to do tests that are not a part of the decision-making process.

Ms. Clarke reiterated that screening guidelines are needed primarily for decisions such as Mr. Miller's "white"/"gray" and "gray"/"black" cutoffs, and for these cutoffs, thresholds based on total PAH may be sufficient. These thresholds would provide the preliminary screen to identify sediment that is "white" or "black" and requires no further testing. For sediment in the gray area, biological testing would always be required. Mr. Miller indicated that guidelines for the "gray"/"black" distinction (upper threshold) could be relatively crude rules-of-thumb, designed simply to spare permit applicants from the unnecessary expense of testing sediment that almost certainly cannot meet unrestricted open water disposal requirements. The "white"/"gray" cutoff (lower threshold) is a more difficult distinction to make, and would require guidelines having strong scientific validity.

Mr. Miller asked what would be needed to create upper and lower thresholds for PAH in southern Lake Michigan sediment. Dr. Mac suggested that a threshold could not be specified using a single total PAH number because of the variability in the action and effects of the individual compounds. Dr. Landrum noted that a single-chemical approach would be extremely complex because each individual PAH would have to be considered in the presence of other chemicals as well. He went on to say that both the upper and the lower threshold would have to be based on a series of biological tests. Dr. Anne Spacie suggested that the thresholds be developed in the same way as the two-number water quality criteria of the USEPA, using site-specific acute and chronic toxicity tests.

Thresholds for PAH in tissues. Ms. Coch asked if threshold values developed for sediment could also be applied to concentrations of PAH in tissues. Dr. Landrum and Dr. Petty both replied that data supporting such an application are for the most part nonexistent. To develop thresholds for an organism, a response spectrum would have to be generated relating tissue concentrations to acute and chronic effects in that organism. Individual response spectra and thresholds would have to be developed for each organism of inter-

est. The participants knew of only one organism for which a response spectrum has been generated, the mussel *Mytilus edulis* exposed to 2- and 3-ring aromatic hydrocarbons from fuel oil (Moore, Livingstone and Widdows 1988; Widdows, Donkin and Evans 1985). Dr. Stein noted that exposures were to PAH in water, but the response spectrum relates biological effects to concentrations of PAH in the mussel tissues. The investigators found that the degree of biological stress to the mussels, as measured by scope for growth, was a direct function of PAH tissue concentrations.

Questionnaire responses concerning criteria and thresholds. Most of the respondents were not aware of any currently existing regulatory criteria governing any of the 15 selected PAH in sediment or tissues (Appendix C, Question B.6). A few criteria have been proposed for PAH in water. The International Joint Commission recommended a water quality criterion of 0.01 micrograms per liter (μg/L) benzo[a]pyrene for the protection of aquatic life in the Great Lakes. The USEPA has developed draft chronic water quality criteria for the following PAH in freshwater:

acenaphthene	57 μg/L
benz[a]anthracene	3.0 μg/L
benzo[a]pyrene	1.2 μg/L
fluoranthene	13 μg/L
phenanthrene	6.3 μg/L
pyrene	13 μg/L

and 4.6 μg/L for phenanthrene in saltwater. Dr. Stainken stated that the US Agency for Toxic Substances and Disease Registry is developing toxicological profiles for benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, dibenz-[a,h]anthracene, and chrysene; these profiles will review international, federal, and state regulatory standards for each compound.

In answer to Question B.4 (Appendix C) concerning a lower threshold for any of the PAH below which that compound is unlikely to have any adverse biological effects, several respondents suggested concentrations of individual or total PAH in sediment or tissues. These were variously reported as PAH concentrations in sediment from "clean" areas, or were considered "no effect" concentrations, SLC, or "background levels." The data are presented in Table 2. Likewise, respondents reported concentrations of PAH in sediment or tissues from "contaminated" areas, or associated with "major biological effects," in answer to Question B.5 (Appendix C). These concentrations are given in Table 3.

Although the values in each table range over several orders of magnitude, the data suggest that "clean" sediment would probably have less than 0.1 ppm of any individual PAH, and "grossly contaminated" sediment would probably have at least 1 ppm of any individual PAH and perhaps several ppm total PAH. These data do *not* establish an unequivocal link between grossly contaminated sediment and biological effects, or between clean sediment and lack of effects. It is quite evident from earlier discussions on residue/effects information that data relating biological effects to levels of PAH in sediment are scant. Furthermore, it would be inappropriate to specify upper or lower thresholds for PAH in sediment from the information in Tables 2 and 3 because the values are derived from a number of different sources under different conditions and for different purposes.

Dr. Dillon warned that there is a large uncertainty factor in trying to predict potential impact. He and other participants intimated that using numeric guidelines for individual chemicals to regulate disposal of sediment containing complex mixtures of chemicals could seriously over- or underestimate impact, due to synergistic or antagonistic interactions. Dr. Dillon suggested evaluating *relative* differences by comparing PAH concentrations in project sediment to concentrations in sediment at the disposal site and a reference area, rather than to absolute numeric guidelines. Dr. Stein added that reliance on biological tests rather than chemical criteria is necessitated by current lack of understanding of the complex factors influencing bioavailability and toxicity.

Establishing upper and lower thresholds for PAH in tissues is even more problematic than for sediment. In addition to many of the difficulties mentioned above, Dr. R. Lee pointed out that measured tissue concentrations may not reflect the amount of PAH taken up due to metabolism or rapid elimination of the compounds.

Summary. The questionnaire responses, workshop discussions, and majority opinions on effects-based screening guidelines or thresholds may be summarized as follows:

a. Problems abound with trying to derive screening guidelines (thresholds) either for total PAH or for the 15 individual PAH. However, guidelines may be more realistic for total PAH than for individual compounds given current knowledge.

b. Not enough data currently exist to set any thresholds for concentrations of PAH in either sediment or tissues.

Organism response and sensitivity to PAH

Organisms can differ greatly in their sensitivity and response to PAH, as indicated by the answers given to Question C.14 in the preworkshop questionnaire (Appendix C):

> C.14. Briefly and in general, how do the biological effects of PAH differ, qualitatively and quantitatively, among different groups of organisms? Do the species recommended in response to Question B.9 differ from each other in sensitivity to PAH?

A number of general statements can be made based on the response to Question C.14:

a. Bivalve molluscs are good indicators of PAH bioavailability because they accumulate parent (untransformed) PAH compounds in their tissues.

b. Animals that feed at the sediment surface or are deposit-feeders will have maximum exposure to sediment-associated PAH. Among those animals, the ones that have well-developed metabolic capability for PAH, such as some crustaceans, will be most likely to show experience toxicity.

c. Toxic effects are more likely produced by PAH metabolites rather than by the parent PAH compounds. Therefore, organisms such as molluscs that have limited ability to metabolize PAH will generally experience low acute toxicity. However, cellular and subcellular pathology has been described in molluscs exposed to PAH.

d. Exposure to PAH can increase MFO activity in fish and many aquatic invertebrates. MFO induction by xenobiotics has been correlated with reproductive impairment in a few species.

e. One of the main threats to fish of PAH exposure is carcinogenic response.

f. Chronic low-level exposure to PAH is more of a problem than acute toxicity in most PAH-contaminated areas.

Information provided by the respondents concerning responses of groups of aquatic organisms to PAH is summarized in Table 4. Differences in sensitivity to PAH among individual species were not discussed to any extent either during the workshop or in the questionnaire response. However, sensitivity differences to toxicants in general can be substantial, even among congeneric species, and also during different life stages within the same species (Buikema and Cairns 1980).

Mr. Miller asked the participants to speculate what level of the food chain is at most risk from PAH in sediment. The ensuing discussion indicated that there is no simple answer to this question. Dr. O'Connor suggested that among the predators, the first level carnivores feeding on molluscs would likely see the most parent PAH, while top level predators would be ingesting biotransformed compounds. However, the issue is greatly complicated by seasonal and life stage changes in diet in many predators. Dr. Mac mentioned that stomach contents analyses of brown bullheads from the Buffalo River indicate that these fish are getting some dietary exposure to parent PAH.

Mr. Miller said he was particularly concerned about the population structure of the Great Lakes sport fishery. Dr. Landrum stated that most Great Lakes fishes will feed on *Pontoporeia* at some point in their life cycle. Because these amphipods are good bioaccumulators of PAH,[7] fishes feeding on them may have dietary exposure to the parent PAH. Dr. Spacie added that the movement patterns of migratory fishes in Lake Michigan will probably bring them past the more highly contaminated spots near the southern end of the lake, where they would have some environmental exposure to PAH. Fishes receiving doses of PAH, either from diet or from other exposure, will biotransform them within days, and the parent compounds will disappear within 48 to 72 hrs, according to Dr. O'Connor. However, some metabolites will be retained and will accumulate in almost all tissues (Gmur and Varanasi 1982; Goddard, Schultz and Stegeman 1987; Jiminez, Cirmo and McCarthy 1987; Moese and O'Connor 1985; Schnitz, Squibb and O'Connor 1987; VanHofe and Puffer 1986). A similar phenomenon may occur in biotransforming invertebrates. Blue crabs, for example, retain 30 percent of the ingested dose of parent PAH, at least of phenanthrene, as transformed metabolites.

Ms. Coch asked whether there is a difference in response to PAH between freshwater and saltwater organisms. Dr. Dillon ventured that the difference in response between freshwater and saltwater organisms will be small relative to the differences in effects due to dissimilarity among the compounds and their analytical variability, and to dissimilarities among species. Nonetheless, biological testing for regulatory evaluation of contaminated saltwater

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7. The New York District emphasized that amphipods would not be a good choice for New York District PAH bioaccumulation testing because of low biomass and difficulty of obtaining specimens in winter. Organisms recommended for bioaccumulation testing are discussed in the sections on Bioaccumulation (p. 43) and Recommendations for a Tiered Testing Approach (see p. 57).

sediments should focus on saltwater organisms, while testing of freshwater sediments should focus on freshwater species.

PAH Biological Testing

In his questionnaire response, Dr. Mac stated, "Because of the number of PAH compounds, their complexity and potential interactions, regulatory testing for PAH contamination in sediment should stress biological testing. It does not appear conceivable that realistic chemical criteria can be established for a number of PAH compounds. Regulatory decisions will have to be made based on bioassessment." These statements succinctly summarize a judgment that was echoed repeatedly by other participants during the workshop discussions. Dr. Mac went on to stress that bioassessment include either a series of laboratory tests, using several organisms and measuring several endpoints, or a combination of laboratory tests and field assessments.

The following sections discuss bioassessment procedures that have potential utility for assessing biological effects of PAH in sediment. These procedures include individual tests as well as approaches that combine tests with numeric guidelines or other information such as benthic community evaluations. To avoid confusion in terminology, the following definitions are taken from Rand and Petrocelli (1985):

> **Bioassays** are tests used to evaluate the relative potency of a chemical by comparing its effect on a living organism with the effect of a standard chemical preparation on the same type of organism.
>
> **Toxicity tests** measure the degree of response (i.e., adverse or toxic effect) produced in an organism by exposure to a specific concentration of a chemical.
>
> **Acute toxicity tests** measure rapid response (usually lethality) to an exposure, generally in 4 days or less.
>
> **Chronic toxicity tests** measure the effects of continuous, long-term exposure of a chemical or other potentially toxic material on organisms. The biological response measured may be lethality or some sublethal effect.
>
> **Bioaccumulation tests** measure the amount of a chemical in organism tissues, taken up from water directly or through consumption of material containing the chemical.

Acute and chronic toxicity tests are bioassays only if they include comparison to a standard toxicant. The Ames test, for example, is a bioassay, but most

of the tests described in the following sections are not. Bioaccumulation tests generally would not be bioassays.

Most of the tests to be described are sediment tests, i.e., they expose organisms to sediment or to some aqueous extract of the sediment. An exposure to whole sediment is a solid phase test. A suspended particulate phase test is an exposure to the unfiltered elutriate (aqueous extract) from the sediment. A water column test uses filtered elutriate. A pore water test uses interstitial water that has been separated from the sediment by centrifugation or expression. Certain tests such as the Ames bioassay employ an organic-solvent extract of the sediment.

Recommendations for specific tests of biological effects due to PAH were solicited in Question B.9 of the preworkshop questionnaire:

> B.9. What specific biological tests would you recommend as tools for assessing toxicity and other adverse biological effects? What species would you use?

The responses to this question are integrated with the workshop discussions as summarized in the following paragraphs. It should be emphasized that many of the tests mentioned in the following sections are experimental and are not currently appropriate in a regulatory context. The approach that *is* considered appropriate for regulatory evaluations at the present time is detailed in the section, Recommendations for a Tiered Testing Approach (p. 57).

Acute toxicity

Although the need for PAH acute toxicity tests was established during the 1986 PAH workshop and reaffirmed early in this second workshop, there was little discussion of specific test procedures. Organisms recommended by the questionnaire respondents for acute toxicity tests include polychaetes, *Daphnia*, *Ceriodaphnia*, fathead minnows, the amphipods *Rhepoxynius* and *Pontoporeia*, and mallard eggs. A different type of acute toxicity test that has potential application is the Microtox test, which uses bacterial bioluminescence rather than lethality as an endpoint. The Microtox test can be performed rapidly and at relatively low cost; however, Dr. Dillon emphasized that there are major problems in adapting the Microtox test for sediment evaluations because it uses an extract (organic solvent or saline) of the sediment (see discussion of problems in Test Media section, p. 63). Dr. Landrum commented that the Microtox test can be used successfully with interstitial water as the toxicant source (Giesy et al. 1988). Dr. Stainken warned that interpretation of

Microtox test results is extremely difficult, and that linking of the test results to toxicity in higher organisms is questionable.

Bioaccumulation

The questionnaire respondents recommended bioaccumulation tests using sediment-dwelling organisms that have limited ability to biotransform PAH. Suggested animals include the bivalves *Yoldia* and *Macoma* for saltwater, and the Great Lakes amphipod *Pontoporeia hoyi* for freshwater. Exposures should be to the solid phase (i.e., whole or deposited sediment). Dr. Mac tentatively recommended the earthworm, *Lumbricus terrestris*, for freshwater bioaccumulation testing (Mac and Willford 1986, Mac et al. 1987). Although the earthworm is not an aquatic organism, it is a deposit feeder and survives nicely underwater in oxygenated sediment. However, the MFO system is present in earthworms and the extent of their ability to biotransform PAH is uncertain (Stenersen 1984). In any case, earthworms are not an appropriate surrogate for species inhabiting an aquatic disposal site.

Ms. Coch and Mr. Miller expressed a concern that benthic organisms bioaccumulating PAH are not biotransforming or metabolizing these compounds, and thus bioaccumulation may not be indicative of adverse effects. How meaningful, then, are tests for bioaccumulation of PAH? Dr. Landrum replied that bioaccumulation demonstrates bioavailability. Bioavailability is a prerequisite for contaminant-related biological impact. Dr. Stainken recommended evaluating bioavailability as a first-cut decision concerning the probability of adverse biological effects. In other words, if molluscs are bioaccumulating PAH from their environment, then the PAH are bioavailable and other organisms capable of metabolizing PAH are also being exposed to the compounds, taking them up, biotransforming them, and potentially suffering adverse effects. In these organisms, however, it is not possible to look for the parent compounds as an indication of the level of exposure. Nor is there enough information at present to equate PAH concentrations in tissues of bioaccumulating organisms with adverse effects in other organisms.

Dr. Weinstein questioned why it would not be possible to analyze for metabolites, since generally the metabolites rather than the parent compounds are responsible for chronic adverse effects. Dr. Petty replied that there may not be enough biomass to analyze for metabolites, and all of the metabolites are not known. Dr. O'Connor stated that there are dozens of metabolites for benzo[a]pyrene alone, and that many PAH metabolites are too unstable to be

reliably detected in chemical analysis. The issue of PAH metabolites was discussed at length during the 1986 PAH workshop and is summarized in Clarke and Gibson (1987a).

Ms. Coch inquired whether there are any quick tests for bioavailability. Dr. Mac reiterated that bioavailability is normally inferred from a bioaccumulation test, and Dr. Tatem said he was not aware of any bioaccumulation test shorter than 10 days. However, he suggested that it might be possible to enhance the uptake of contaminants and thus shorten the duration of the test by continuously exposing the organisms to suspended sediment.

Potential for bioaccumulation of PAH from sediment can be estimated quickly for nonmetabolizing organisms using a method developed at the WES (McFarland 1984; McFarland and Clarke 1986, 1987). The method is based on thermodynamic principles and applies to neutral organic chemicals such as PAH. It requires only knowledge of sediment organic carbon content, PAH concentrations in the sediment, and organism lipid content. A simple equation calculates bioaccumulation potential in the lipids of the organism from the organic-carbon normalized sediment contaminant concentrations.

A bioaccumulation test demonstrates only whether the analyzed contaminants are bioavailable. Therefore, Dr. Landrum suggested that a bioaccumulation test might not be necessary if adequate sublethal effects tests are conducted. Dr. Peddicord explained that current regulations require evaluating the effects of pollutant transfer and concentrations through biological processes. This has always been interpreted to mean measuring body burden of the contaminants (i.e., via a bioaccumulation test). However, it might not be necessary to measure body burden if sublethal effects tests are performed instead. For example, if sublethal effects were assessed and found not to occur, the body burden could be considered acceptable even though it was not directly analyzed. However, Dr. Landrum warned that before sublethal effects tests could be substituted for bioaccumulation tests, it would be necessary to demonstrate that exposure produces the effect being assessed.

Tests for sublethal effects

Dr. Tatem suggested beginning the regulatory evaluation with a series of chemical analyses and an acute toxicity screen to determine whether more testing is required. This approach corresponds to the Tier I testing recommended at the 1986 PAH workshop. Mr. Miller responded that he is concerned about the "gray" sediment, when he knows that further testing beyond Tier I will be

needed, but does not know which sublethal effects tests will be most appropriate or useful. He pointed out that there are many different tests available, and requested guidance concerning which one(s) to choose for regulatory evaluation of PAH-contaminated sediment.

In particular, Mr. Miller asked whether some of the sublethal effects tests may be more applicable than others to test for effects due primarily to PAH. Dr. R. Lee replied that there are relatively specific biological responses to certain classes of pollutants, but that it is necessary to know the mechanism of response before determining the best test to use. Dr. Landrum suggested that using a bioassessment test or suite of tests is a more integrative approach than trying to regulate based on numeric criteria, because the bioassessment test does not necessarily attempt to tie a specific response to a specific compound or concentration. Furthermore, the bioassessment test is best used to observe an integrated response of the test organisms to the whole complex mixture of compounds in the sediment, rather than to one particular compound or class of compounds. Specific tests recommended by various participants during the workshop discussions or in their questionnaire responses are described in the following sections. Dr. Dillon emphasized that more work is needed prior to regulatory implementation of any of these sublethal effects tests.

Growth and reproduction. To minimize controversy, Dr. Spacie recommended using a test specifically for reproductive effects or a growth test that includes a reproductive component. The ability to reproduce successfully is an indication of fitness in the individuals and population. The concerned public, for example, may more readily accept reproductive success than other, seemingly more esoteric biological endpoints, as a sign of environmental health. Dr. Peddicord observed that regulatory agencies are also placing more and more emphasis on reproductive effects, and thus reproductive bioassessments are likely to become increasingly important in the future.

Several participants suggested using various partial life cycle tests to assess effects of PAH on growth and reproduction. A partial life cycle test, as described by Dr. Landrum, would expose organisms to contaminated sediment before they enter their reproductive phase, and then would follow them through reproduction. Various reproductive endpoints might be examined, such as number and survival of offspring and their ability to develop normally.

A *Ceriodaphnia* (zooplankton) test for reproductive effects was mentioned. Mr. Adams noted that *Ceriodaphnia* is native to the Great Lakes,

reproduces in a relatively short period of time and has a wide range of sensitivities to contaminants. However, it is quite difficult to rear in the laboratory. Dr. Mac also cautioned that the *Ceriodaphnia* test is not truly a sediment test. Dr. Tatem suggested that a reproductive effects test could be done using *Daphnia magna*, which reproduces in 10 to 12 days (starting with 4-day old animals) and is easy to culture. However, *Daphnia* is also a water column organism. Dr. Fredette indicated that the saltwater mysid shrimp *Mysidopsis* has a short life cycle and might be suitable for a partial life cycle assessment of reproductive effects though it also is not a benthic infaunal organism. Dr. Peddicord suggested that a 7-day rapid-chronic test using mysids in small beakers with deposited sediment might force the mysids to have sufficient exposure to the sediment. Ms. Coch asked whether the mysids will accumulate the 15 selected PAH, and how much tissue would be needed to analyze for them. Dr. Petty replied that it would be possible to measure the 15 PAH in the mysid tissues if there is sufficient biomass. Information subsequently provided by the New York District indicated that the amount of biomass needed for a mysid bioaccumulation test would be cost-prohibitive, requiring more than 100 mysids per replicate.

Dr. Peddicord noted that the saltwater amphipods *Ampelisca* and *Rhepoxynius* are much more intimately associated with the sediment than *Mysidopsis*, but cannot presently be cultured successfully in the laboratory. *Rhepoxynius* appears sensitive to grain size (DeWitt, Ditsworth and Swartz 1988). Isopods, both freshwater and saltwater species, might be good candidates for partial life cycle tests, according to Dr. Weinstein, because they are easily obtainable for a good portion of the year and can be held in the laboratory for lengthy periods of time.

Dr. Peddicord also described a sediment pore water test using the archiannelid *Dinophilus*, which has a life cycle of about 7 days, and is sensitive to environmental contaminants. In this whole life cycle test, mortality, development, growth and reproduction can all be assessed. However, the test was devised for effluent evaluation and has not been adapted to include sediment because the egg capsules are too difficult to find in the presence of sediment.

Dr. Ross described a rapid reproductive test using larvae of the nematode *Pannogrellus* in pore water, with percentage of gravid females as the test endpoint. The adult stage is achieved in 96 hours following four molts. Individuals in each stage have a distinct, recognizable size. Under adverse

conditions such as lack of food or water, individuals become dormant and can remain so for many years until more favorable conditions return. This characteristic enables them to be easily kept and stored in the laboratory. Nevertheless, the species apparently is quite sensitive to contaminants, and Dr. Ross has used *Pannogrellus* extensively in acute toxicity tests. Lethality is easily determined because the integument is only two cell layers thick and the worm disappears within one minute of death. Unfortunately, the test is not easily adaptable to solid phase because these microscopic animals are too difficult to see in sediment.

Dr. Weinstein recommended that any test for biological effects of contaminants in sediment should employ organisms that live in a sedimentary environment. Dr. Fredette added that, for regulatory evaluation of dredging and disposal, the test organisms should be organisms living at the disposal site, or surrogates for them, if the disposal site species present particular collection or culture problems that render them impractical for laboratory testing. Dr. Landrum lamented that animals well suited for laboratory tests are frequently not those that will be found at the disposal site. Dr. Weinstein intimated that exact correspondence in species between laboratory test and disposal site is not necessary if the test organisms employed are sufficiently sensitive to represent those present at the disposal site. Ms. Coch contended that organisms currently used by the New York District for acute toxicity and bioaccumulation in the solid phase testing program (CE/USEPA 1984), namely the grass shrimp *Palaemonetes pugio*, the clam *Mercenaria mercenaria*, and the polychaete *Nereis virens*, are representative of species living at the disposal site.

Most participants agreed that sediment tests for chronic effects should include some assessment of reproduction, such as a partial life cycle test. Unfortunately, all of the possiblities mentioned above for reproductive bioassessments to be used in the regulatory evaluation of dredged material can be criticized for one or more of the following reasons:

a. Animals are not associated with the sediment (*Ceriodaphnia*, *Daphnia*, *Mysidopsis*).

b. Animals may be associated with the sediment but individuals or egg capsules are too small to be seen in the presence of sediment (*Dinophilus*, *Pannogrellus*).

c. Animals are associated with the sediment but do not reproduce successfully in the laboratory (*Ampelisca*, *Rhepoxynius*).

d. Animals may be associated with the sediment but standardized reproductive bioassessments have not yet been developed (isopods).

Dr. Spies also cautioned that more work is needed to demonstrate that positive results can be achieved from controlled laboratory experiments to assess reproductive effects of PAH.

Given the absence of reproductive bioassessments that could be included at present in the Corps regulatory program, Dr. Dillon proposed generating a "dose-response" database for PAH, in which tissue concentrations of PAH (dose) are coupled with observed reproductive effects (response). Thus, some defined low level of PAH in tissues could be associated with low response in terms of adverse effects on reproduction, moderate tissue PAH levels could be associated with moderate response, and high tissue PAH levels with high response. This categorization could be combined with the matrix approach for regulatory evaluations. Dr. Landrum objected that there are currently no data to generate such a database, and that it would be difficult or impossible to determine dose-response relationships for PAH because of metabolism. Most of the adverse effects are likely to stem from the metabolites rather than the parent PAH, and there are too many metabolites to realistically measure them. Organisms with limited ability to metabolize PAH are not likely to experience the same level of effect as those that do metabolize PAH. Dr. Stein stated that this approach has been used with *Mytilus edulis*, but the dose-response relationship generated for this mussel is not directly applicable to other organisms. Dr. R. Lee indicated that dose-response relationships can be determined for reproductive effects in at least one species of fish using a molecular biology approach that measures cytochrome P-450 induction (explained in the section Enzyme induction and reproduction, p. 49).

Dr. Dillon suggested calibrating effects in metabolizing animals with tissue concentrations of PAH in nonmetabolizing animals. Calibrating effects with tissue residues rather than with sediment concentrations would link effects with bioavailability. It would also eliminate the necessity of doing the calibration in every dredging project evaluation. Dr. Spies cautioned that it would be necessary to determine if the instantaneous rate of uptake is similar in the metabolizing and the nonmetabolizing animals. Similarity in PAH uptake rates would be a necessary prerequisite for Dr. Dillon's calibrations. Mr. Vic McFarland said that determination could easily be done using radiotracers.

Dr. O'Connor raised a general objection to reproductive bioassessments, in that it could be difficult to obtain good results from the contract laboratories that would have to perform these tests for regulatory evaluations. Moreover, reproductive effects on populations may be relatively minor due to dilution in large aquatic systems receiving comparatively small amounts of dredged material and contaminants. Dr. O'Connor favored bioassessment tests for growth rather than reproduction because he felt they could be more readily conducted by researchers and potentially by contract laboratories, particularly with fairly easy-to-maintain animals such as some amphipod species. He noted that growth tests using relatively large organisms could be done in a reasonably short period of time, and would provide sufficient biomass to investigate questions concerning enzyme induction or gene amplification. Suitable organisms might be small fishes such as fathead minnows in freshwater, or the sand dab *Citharicthys* (a west coast flatfish) or *Menidia* (silversides) in saltwater. The test would start with embryos or larvae, stages in which growth and morphological changes occur rapidly. Reproductive tests, on the other hand, using these species could be prohibitively long. The tendency to develop rapid reproductive assays necessitates the use of smaller and smaller organisms, which makes it difficult to employ a suite of tests, because not enough biomass is available from the reproductive test to conduct other tests.

Mr. Adams proposed a chronic, freshwater, sediment test for growth reduction in *Chironomus tentans*. He described the 10-day test as sensitive, useful, and relatively inexpensive, employing animals that are easy to grow and are native to the Great Lakes. Data on sediment from the Detroit River suggest that greater than 30 percent growth reduction in *C. tentans* relative to a control is an indication that there will be no chironomids living in the sediment (Giesy et al. 1988).

Enzyme induction and reproduction. Dr. Spies stated that he has had success in relating a biochemical endpoint, cytochrome P-450E induction, to reproductive effects in starry flounder (*Platichthys stellatus*), a Pacific coast flatfish. Cytochrome P-450 is the terminal oxidase in an enzyme system involved in the biotransformation of PAH, and P-450E is one of several related enzymes of this system. The level of P-450E activity was measured in female starry flounders right after spawning. Correlation of P-450E induction with reproductive effects has been demonstrated in spot (*Leiostomus xanthurus*) as well as starry flounder. Dr. R. Lee explained that little or no cytochrome P-450E is present in fish tissues under normal circumstances and the level of

this enzyme markedly increases when the fish is exposed to a PAH or PAH-like compound such as certain PCB congeners. Induction occurs quickly, within a matter of days, in response to contaminant exposure, and gradually disappears when exposure ceases. P-450E can be assayed using a monoclonal antibody. The assay is quick and hundreds of samples can be tested in a day. Dr. R. Lee felt that the test could easily be performed by any contract laboratory capable of doing a lysate test.

The cytochrome P-450 induction test has potential utility because it examines a response that is specific to a class of pollutants, and dose-response relationships can be generated at environmentally realistic levels. P-450 induction may be linked to reproduction, because the P-450 enzymes function in the processes leading up to reproduction, namely the metabolism of reproductive steroids. Thus the P-450 induction test is able to correlate levels of an enzyme with lack of reproductive success, at least in certain fishes. P-450 induction is also implicated in carcinogenesis in some fish species and in mammals, according to Dr. Spies.

Several participants expressed reservations about recommending the P-450 enzyme assay for inclusion in a regulatory testing protocol. Dr. Stein commented that induction of P-450 is a sensitive indicator of exposure to anthropogenic chemicals, many of which are potentially toxic. It is a good indicator of exposure to low doses of PAH, for example, but is not responsive to high doses (Collier and Varanasi 1987). The correlations between P-450 induction and reproductive effects or carcinogenic/mutagenic effects have been demonstrated in only a few species. Moreover, whether induction of P-450 enzymes is *directly* related to these effects is not known. Dr. Dillon also noted that P-450 induction can be substantially affected by factors such as season, feeding, and sex of the individual.

Carcinogenicity/mutagenicity. Mr. Miller inquired about the status of tests for mutagenicity or carcinogenicity of PAH, either in isolation or in conjunction with other sediment contaminants. He asked whether the Ames test might be an appropriate procedure to use. The Ames test uses a centrifuged liver homogenate supernatant as an activating agent for bacterial mutagenicity, as measured by number of revertant bacterial colonies. Dr. R. Lee, in his questionnaire response, noted that the Ames assay has been used successfully with the PAH fraction of sediment extracts (Reilly, O'Connor and Boone 1986). An alternative procedure is to inject sediment extracts into fish and then use their liver homogenates in the Ames test (Kurelec et al. 1979).

Dr. Mac indicated that the Ames test generally produces reliable positive results, but there is not have much confidence in the negative results, i.e., the test can give false negatives (Tennant et al. 1987). Furthermore, it may not be suitable for testing mixtures. In generating a dose-response from serially diluted extracts of sediments from five Great Lakes harbors, better results were obtained from the Chinese hamster ovary assay than from the Ames test (Fabacher et al. 1988). Dr. Mac emphasized that either test can only indicate a *potential* for mutagenicity or carcinogenicity, that the tests do not demonstrate bioavailability, and that it is difficult to interpret the significance of an observed dose-response. Dr. Dillon proposed a more direct evaluation of carcinogenicity using small aquarium fish exposed directly to sediment (see Mix 1986), but Dr. Stein commented that such a test would be expensive.

Other sublethal effects. Respiration in grass shrimp appears to be a sensitive physiological index of sublethal stress associated with exposure to contaminated sediment. Alden, Butt and Young (1988) found that respiration rate was depressed in *Palaemonetes pugio* exposed to the suspended solids fraction of dredged material contaminated with PAH and heavy metals. Under the Corps Field Verification Program, Johns and Gutjahr-Gobell (1988) concluded that respiration rate was depressed in the polychaete *Nephtys incisa* exposed in the field to suspended and bedded dredged material at an offshore disposal site. The dredged material was harbor sediment contaminated with PCB, PAH, and heavy metals. Dr. R. Lee, in his questionnaire response, mentioned an association between cataract development in fish (croakers) and high PAH concentrations (Huggett, Bender and Unger 1987). Dr. Clifford Rice noted that malformities in mallard eggs can be an indication of embryotoxicity associated with PAH exposure.

Summary. In the workshop discussions it became obvious that there is no single, specific, sublethal effects test or even a suite of tests that all of the participants could agree is the best procedure to use at this time. This lack of consensus reflects the state-of-the-art because development of sublethal effects tests using sediment is in its infancy. The participants did agree that any single test, either chemical analysis of sediment or a bioassessment, cannot adequately address the bioavailability and potential acutely toxic or chronic sublethal effects of PAH in sediment. Clearly a suite of tests is needed. However, Dr. Spies cautioned that adding more tests indiscriminately does not necessarily produce a clearer picture of the environmen-

tal significance of PAH. He remarked that a series of tests conducted on San Francisco Bay sediments, including five or six toxicity tests, two bioaccumulation tests, and chemical analyses, resulted in a poor correlation between any toxicity endpoint, bioaccumulation, and any chemical data.

Mr. Miller asked if the participants could identify the characteristics of a good suite of sublethal effects tests. Dr. Landrum said that the tests must be sensitive to the contaminants in the dredged material to be regulated. Dr. Mac recommended that the tests be site specific to the extent that they assess the particular impacts known or suspected to occur in the dredging and disposal area. However, the tests cannot be compound specific because there are hundreds of compounds in sediment that can potentially cause adverse effects. Dr. Peddicord cautioned against using inappropriate organisms, e.g. bivalves in an acute toxicity test. Dr. Spies added that good tests must not introduce artifacts. For instance, *Rhepoxynius abronius* is sensitive to contaminants but is also sensitive to grain size, and thus could show a response to unsuitable grain size rather than to contaminants in the tested sediment. In other words, it is important to choose an appropriate reference sediment, as well as test organisms that are appropriate for the physical characteristics of the sediment.

Dr. Fredette summarized desirable characteristics of sublethal effects tests as follows:

a. Quick.

b. Inexpensive.

c. Use benthic infaunal organisms that are easy to culture and representative of species at the disposal site.

d. Assess life cycle effects (growth and/or reproduction).

e. Produce results that can be related to field organisms and impacts.

Comparison to disposal site

Introduction. Dr. Mac suggested that reference sediment from the disposal site can be used as a control value in the toxicity tests, and Dr. Charles Lee agreed that this is a good approach. Ms. Coch indicated that this is similar to the approach currently in use by the New York District. Percent survival of *Palaemonetes*, *Mercenaria*, and *Nereis*, exposed to dredging project sediment, is statistically compared to percent survival of the same species exposed to a reference sediment. Bioaccumulation in animals surviving

the 10-day test is also compared between project and reference sediment exposures. The reference site is an area 2.5 miles offshore that is ambient "clean" (animals are living there with no apparent problem). The comparison of project to reference is intended to prevent further degradation to the environment, taking into account the fact that the New York Bight experiences ecological stress from a variety of sources.

Dr. Landrum agreed with Dr. Mac that comparing project to reference is a good place to start. However, he cautioned again that bioaccumulation test results must be based upon organisms having very limited ability to biotransform PAH. Mr. Miller asked whether the comparison of project to reference should be based upon bioaccumulation or upon bulk sediment chemistry. Several participants concurred that both bioaccumulation and sediment analysis should be used in the comparison.

Statistical comparisons. The subject of interpreting statistical significance in comparing reference with project dredged material was discussed briefly. Dr. Landrum stated that assessing the magnitude and significance of statistical differences is the job of the regulator. Dr. Dillon emphasized that statistics are only a means to help in regulatory decision making. The decision must be made in the context of the particular dredging project, disposal alternatives, and environmental protection goals.

Mr. Miller asked if all of the reference data generated over the last few years can be combined and used as a single baseline number in the statistical comparisons, instead of including a reference sediment and generating a new reference value in every test. Dr. O'Connor responded that sample sizes for dredged material and reference must be reasonably similar for realistic comparisons. Using hundreds of data points to generate a reference value, for example, would result in a standard error for reference far smaller than the dredged material standard error based on, say, three samples, given the same range of values for the two sediments. In this situation, virtually any dredged material would be statistically different from the reference, and nothing would pass for unrestricted open water disposal.

The matrix approach

Ms. Coch inquired whether determination of ambient body burdens for PAH might be a viable approach for regulatory evaluation of PAH-contaminated sediment. She indicated that data from which these ambient values might be generated for PAH will be collected during the next year. Data are available on

PAH parent compounds in tissues from New York Bay and Bight beginning around 1976, according to Dr. O'Connor, but the analyses were performed by many different laboratories and no standard analytical protocol was used. Ms. Coch noted, however, that all analyses performed for the New York District were done using standard protocol specified in the USEPA/CE Guidance for Performing Tests (CE/USPEA 1984). Dr. Landrum pointed out that using a matrix approach for PAH would at least be consistent with the approach used by the New York District for PCB, cadmium, and mercury. Dr. O'Connor explained that the ambient values for those contaminants were derived by calculating, using pharmacokinetic principles, the amount of PCB in *Nereis*, for example, that could result in a body burden of 2 ppm PCB (the Food and Drug Administration (FDA) action level) in fishes feeding on those worms. Furthermore, it was observed that in areas of the New York Bight where fish food organisms did not exceed the ambient value for PCB, their respective fish predators rarely, if ever, exceeded the FDA action level for PCB. However, the matrix approach cannot be applied to PAH, according to Dr. O'Connor, because fishes and many invertebrates quickly metabolize the parent PAH. Moreover, there are no FDA limits for PAH from which to calculate ambient values.

Total versus individual PAH

Dr. C. Lee and Dr. Dillon asked if all of the 15 selected PAH should be treated the same in terms of toxicological importance, or whether a sum of the 15 might be more interpretable in a regulatory context. This was essentially the same question raised earlier under the consideration of numeric regulatory guidelines for PAH. The responses and recommendations given by the participants paralleled those given earlier. Dr. Spies observed that concentrations of the 15 PAH tend to rise and fall as a group. Dr. O'Connor recommended abandoning the oil and grease test, which was also proposed during the 1986 PAH workshop, and measuring "total PAH" as a sum of the 15. The chromatographs for this analysis will contain useful information on the individual compounds and should be saved for future use. Dr. Peddicord added that the current regulatory approach of comparing dredged material to reference sediment should be continued, only using total PAH as the sum of the 15, rather than using oil and grease. This procedure would be analogous to that currently used for PCB, in which "total PCB" is actually the sum of several peaks on the chromatograph.

Combined approaches

Field assessments and laboratory tests. Mr. David Norris indicated that the USEPA is considering different bioassessment tests with the objective of developing a good regulatory approach for the Great Lakes. He, too, emphasized the need for a suite of tests. Mr. Norris favored the sediment quality triad approach of Chapman and colleagues (Chapman 1986; Chapman, Dexter and Long 1987), in which benthic community structure assessments can be used to validate laboratory test results. Dr. Mac, in his questionnaire response, also proposed using the sediment quality triad, or some other combination of laboratory tests and field assessments such as benthic community structure or a fish tumor survey.

Decision making framework. Dr. C. Lee presented a management strategy, known as the decision making framework, which was developed to assist in the interpretation of sediment testing data (Peddicord et al. 1986). The first question asked in the framework is whether there is a reason to believe that the sediment is contaminated. If the answer is yes, the decision making framework will assist in answering the following question:

> What will be the potential environmental impact of dredged material disposal in a particular disposal environment (upland, wetland, or aquatic)?

If the material is to be placed in the aquatic environment, the regulator must consider the impact on the water column and the impact on the benthos. The decision making framework is designed to facilitate the environmental impact evaluation and regulatory decisions concerning disposal options. The framework considers not only test results, but also possible disposal restrictions and the available engineering or operational actions that can be initiated to minimize potential impact.

The framework presents flowcharts for comparing dredged material to reference material in a step-by-step process for sediment chemistry, acute toxicity, and bioaccumulation. Biological test results are compared not only to a reference but also to available guidelines, such as a specified percent mortality for the acute toxicity test, and FDA limits or ambient values for the bioaccumulation test. In the framework, the bioaccumulation test could easily be supplemented or replaced by one or more tests for sublethal effects.

Following the appropriate pathway in the aquatic disposal flow chart may lead the regulator to a decision of unrestricted aquatic disposal, aquatic disposal with restrictions, or to a "local authority decision" (LAD). If faced with a LAD, the regulator or project sponsor could choose to do additional tests in an effort to obtain a more definitive outcome. If the LAD results in prohibition of aquatic disposal, the regulator might then consider other disposal alternatives and repeat the step-by-step evaluation process using the appropriate flowcharts.

The New England Division approach. Dr. Fredette explained that the regulatory approach used by the New England Division differs somewhat from the decision making framework in not stipulating parallel bulk sediment chemistry and biological testing. This approach is more cost-sensitive than the decision making framework in that the early testing tiers utilize the less expensive tests. The first step or tier is a sediment grain size analysis and consideration of the source of the sediment. If the sediment is sand, and/or far removed from known or suspected sources of contamination, no further testing would be required. The second tier is a bulk sediment analysis, in which sediment contaminant levels are compared with screening level criteria used by the State and based on those developed by the New England River Basins Commission. At this point in the approach, approximately 65-80 percent of the dredging projects in the Division can be approved for unrestricted aquatic disposal. The third and most expensive tier involves biological testing (acute toxicity and bioaccumulation). Extensive and long-term monitoring of disposal sites has indicated that biological communities near the disposal mounds (including mounds where capping has been performed) are not adversely impacted by the sediment allowed for ope water disposal. The lack of field effects supports the evaluation decisions and management practices currently in effect.

Relating laboratory tests to environmental impact

The workshop participants generally agreed that at least some of the laboratory tests described above should be able to demonstrate adverse biological effects from exposure to PAH-contaminated sediment. However, this only indicates a *potential* for environmental impact. What happens in the laboratory will not necessarily happen in the aquatic environment. The extent to which environmental impact might be predicted by laboratory test results was addressed in the preworkshop questionnaire (Appendix C):

B.11. Is it possible to relate the results of short-term laboratory tests to impacts from long-term exposures in the aquatic environment?

Several respondents said that this is not possible at the present time. Mix (1988) also cautioned against extrapolating laboratory results to field situations because of the difficulty in distinguishing between toxic effects and background noise in natural populations. Dr. Peddicord noted, however, that adverse effects observed in a short-term laboratory test do indicate "concern."

Dr. R. Lee postulated that MFO induction and cellular/subcellular changes in reproductive tissues may be a way of relating laboratory results to impacts on reproduction. Dr. Mac mentioned that several fish assays, which may be useful in predicting carcinogenicity, are being developed but will require extensive laboratory testing time of 6 months to one year. Such tests thus do not really qualify as short-term laboratory tests, nor are they feasible for regulatory evaluations.

Recommendations for a Tiered Testing Approach

Introduction

At the final workshop session, Ms. Clarke drew together tentative agreements reached during previous sessions, and asked the participants to help flesh out the tiered testing approach for regulatory evaluation of dredged material containing PAH. Ms. Coch had earlier proposed certain guidelines that the New York District would like to see incorporated into this testing approach. First, as she reiterated from the 1986 PAH workshop, the District must utilize commercial laboratories for sediment testing. Thus, the regulatory procedures to be implemented must be suitable for use by these contract labs. Current testing procedures are based upon joint USEPA/Corps guidance, with modifications developed by the District to handle regional problems and conditions (USEPA/CE 1977, CE/USEPA 1984).

Second, the District feels that the tiered testing approach proposed at the 1986 PAH workshop is a good one and should, with the possible exception of bulk sediment chemistry, be used as the basis for more detailed testing recommendations. PAH are probably ubiquitous throughout the New York Harbor, according to Ms. Coch. Because there is already reason to believe that the har-

bor sediments are contaminated with PAH, the New York District is inclined to skip the Tier I sediment analysis recommended by the first workshop. The District does wish to retain its own current testing framework, including a 10-day bioaccumulation test, but would consider adding an additional organism to the three already in use (*Nereis*, *Mercenaria*, and *Palaemonetes*) if there is a compelling reason for doing so. Dr. Tatem, however, objected that these three currently used organisms are not sensitive enough for acute toxicity determinations. Dr. Fredette countered that representation of field effects, not sensitivity, is the prime objective of bioassessments.

The testing approach as derived from the workshop discussions would have three tiers that can be implemented now, and a fourth tier consisting of tests that require additional research or development, and depending on the test, might be implemented in the near to not-so-near future. The approach as formulated did not engender universal agreement or enthusiasm among the participants. However, it did embody the universal frustration of the participants over the current state-of-the-art in PAH research and sediment testing, which are not sufficiently advanced to address the primary needs and concerns of the Districts. The actual guidance proposed at the workshop has been modified slightly herein to conform to the tiered testing approach laid out in the national comprehensive testing strategy supported by the Corps (Engler et al. 1988).

The first three tiers are essentially an elaboration of the tiered testing approach proposed during the 1986 PAH workshop. Tier I is the initial evaluation of existing information and determination of whether there is "reason to believe" that PAH contamination is present.[8] Tier II consists of chemical analysis of the sediment for the 15 selected PAH to determine whether there is reason to believe that the dredged material is more contaminated than the disposal site (or reference) sediment and potential unacceptable adverse effects may occur. Tier III is the biological testing tier and includes acute toxicity tests and bioaccumulation tests.

The acute toxicity tests of Tier III could utilize organisms such as those currently used by the New York District (*Mysidopsis*, *Palaemonetes*, and *Nereis*), or an amphipod such as *Rhepoxynius* or *Ampelisca*, for saltwater. *Rhepoxynius*, however, is thought to be sensitive to grain size and caution is

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8. Tier I, as described here, was included as a preliminary evaluation but was not specifically labeled as a tier in the 1986 PAH workshop tiered testing approach because no actual testing may be involved in this evaluation.

needed in tests where the dredged material and reference sediment differ markedly in grain size. Freshwater organisms appropriate for acute toxicity testing include *Daphnia*, *Ceriodaphnia*, or algae such as *Selenastrum* for elutriate or pore water testing, perhaps in combination with a Microtox test. Larval fathead minnows, *Chironomus*, or larvae of the mayfly *Hexagenia* are appropriate for solid phase acute toxicity testing.

The Tier III bioaccumulation tests must use organisms that have limited capacity to metabolize PAH. Suggested species include the deposit-feeding bivalves *Macoma* and *Yoldia* for saltwater, and the amphipod *Pontoporeia* for the Great Lakes. Filter-feeding bivalves are not recommended for this test. Species of *Macoma* are very hardy, and will survive and bioaccumulate in sediment that is toxic to more sensitive organisms. *Pontoporeia* is indigenous to the Great Lakes, lives in a wide range of sediment types from coarse sand to fine silt, and bioaccumulates relatively high levels of neutral organic chemicals because of its high lipid content. However, *Pontoporeia* has not been cultured successfully in the laboratory and must be collected from the Great Lakes, which is difficult during the winter. Also, this amphipod is not hardy unless it is kept cool (4°C) and in the dark. Dr. Landrum suggested that *Hexagenia limbata*, which has very low biotransformation capability for PAH (Landrum and Poore, in press), would be a suitable freshwater species for bioaccumulation testing in warmer regions. *Chironomus* was also proposed as a possible organism for freshwater bioaccumulation tests. However, Dr. Landrum indicated that *Chironomus riparius* readily biotransforms benzo[a]pyrene (Leversee et al. 1982) and anthracene (Gerould, Landrum and Giesy 1983), and that other chironomid species are likely to have the capability for PAH biotransformation but would need to be tested for this capability before being recommended or ruled out for bioaccumulation studies.

The participants stated repeatedly during the workshop that it is not currently advisable to specify numeric guidelines or thresholds for regulation of PAH-contaminated sediment. Thus, the significance of both Tier II and Tier III testing would be determined by comparing test results from dredging project sediment to results from an appropriate reference sediment. Ideally, the reference sediment should come from an area near or similar to the disposal site. If the project test results are statistically significantly greater than the reference test results, then there is the potential for unacceptable adverse biological impacts to occur as a result of dredging and disposal operations.

At present there is little information relating concentrations of the 15 PAH in sediments or in tissues to biological effects, or assessing the relative toxicities of the 15 compounds. Thus it may be difficult to interpret Tier II and Tier III bioaccumulation results for the individual PAH. For example, if one PAH is significantly higher in the dredged material than in the reference sediment but the other 14 PAH are not, is this basis for "failure" of the sediment analysis? Until a database can be developed relating environmental concentrations of the individual PAH with biological effects, an interim recommendation would be to compare dredged material and reference test results for "total PAH" as a sum of the 15, as proposed earlier in the Total versus individual PAH section (p. 54). Using this approach would have the advantage of generating values for the 15 individual PAH that could be incorporated into the database, but would not at present be used for regulatory decision making.

Ms. Coch asked whether the organisms for biological testing should be collected from the dredging site and reference site. The participants pointed out several difficulties with that approach. First, the same species might not be found at both sites. Second, it could be difficult to collect enough biomass for tissue analyses. Finally, populations living at the dredging site could be adapted to relatively high concentrations of contaminants, and might not show the same level of effects as organisms that had not been chronically exposed to the contaminants.

The tiered testing approach for regulatory evaluation of PAH-contaminated dredged material, as outlined above, corresponds with Tiers I-III of the Corps' comprehensive testing approach for aquatic disposal as part of the Federal Standard. This approach is explained succinctly by Engler et al. (1988):

> The national comprehensive testing strategy supported by the Corps is a tiered approach with each successive tier being based on a "reason to believe" that there is potential for unacceptable adverse effects. Each tier is fully optional and may be subsequently eliminated if there is sufficient information available to provide an adequate assessment for that tier or if there is no reason to believe that there will be unacceptable adverse effects associated with that tier or disposal concern. Such multiple tests are clearly allowed by 40 CFR 230.4-1 ("No single test or approach can be applied in all cases to evaluate the effects of proposed discharges of dredged or fill material," and "Suitability of the proposed disposal sites may be evaluated by the use, where appropriate, of sediment analysis or bioevaluation."). However, such tests are subject to the condition that "In order to avoid unreasonable burdens on applicants in regard to the amounts and types of data to be

provided, consideration will be given by the District Engineer to the economic cost of performing the evaluation, in light of the information expected and the contribution of that information to the final decision, and the nature and magnitude of any potential environmental effect."

The first tier of the existing approach consists of an initial evaluation of available information to establish whether there is a "reason to believe" that contaminants are or are not present. This tier is commonly referred to as the "exclusion clause" (40 CFR 230.4-1(b)(1)). If there is no reason to believe that contaminants are present and if certain other conditions are met, including grain size and chemical/physical similarity of the dredged material and the substrate at the disposal site, no further testing is required. If there is reason to believe that contaminants are present, or if sufficient information is not available, a second tier or evaluation may be conducted which consists of a bulk sediment analysis. Should sufficient information be available from previous testing and evaluation, no additional chemical analyses are necessary.

The bulk sediment analysis is essentially an inventory of contaminants of concern and is used to compare the chemical composition of the dredged material to the composition of the material at the disposal site with emphasis generally placed on heavy metals, PCBs, PAHs, pesticides, and other substances of ecological or human health significance. If substantially greater concentrations are observed in the dredged material and there is reason to believe that the substances are bioavailable and sufficient information is not available, a third tier of testing may be required. This tier includes testing for water column impacts and/or benthic impacts.

If there is concern regarding water column impacts, an elutriate test may be performed to evaluate contaminant release into dredging or disposal site water. The results of the elutriate test are compared to water quality standards after consideration of mixing as described in the 404(b)(1) Guidelines. If there are no water-quality standards or the standards are thought to be inappropriate or inadequate, a water column liquid and/or suspended particulate phase bioassay may be conducted along with consideration of mixing. Again, depending on where the concern lies, the water column bioassay may address the dissolved constituents and/or the suspended solid particulate phase.

If there is concern regarding impacts to the benthic organisms, a benthic bioassay may be conducted. In general, for a comprehensive assessment of potential impacts, three organisms are generally used: a filter-feeder, a deposit-feeder, and a burrowing species. These relate to potentially different ecological niches at the disposal site. In addition, a mysid shrimp may be considered and has been widely used as an internal standard and to form a basis for quality assurance.

If there is reason to believe that bioaccumulation is of concern, a second component of the third tier consists of evaluating the potential uptake of contaminants. This may be done either in the field or in the laboratory, whichever is more appropriate. If done in the laboratory, it is customary to use survivors of the toxicity bioassays for bioaccumulation assessment if sufficient biomass is present in the survivors.

Tier IV of the testing approach proposed at the workshop is the sub-lethal effects assessment. This tier would include an evaluation of the

potential for adverse impact on reproduction and growth. One recommended bioassessment is a partial life cycle test using organisms such as *Mysidopsis* in saltwater, and fathead minnows, chironomids, the amphipod *Hyalella*, or *Daphnia magna* in freshwater. Mr. Norris mentioned that the American Society for Testing and Materials has a draft protocol out for 28-day chronic tests using *Hyalella* and *Chironomus*. Dr. Mac pointed out that the 28-day *Chironomus* test involves growth and maturation, but not reproduction. *Daphnia magna*, although not a benthic organism, can be useful for assessing effects due to volatile components in resuspended sediments or pore water. As in Tiers II and III, test results for organisms exposed to dredging project sediment would be compared to results for organisms exposed to an appropriate reference sediment to determine test significance.

An assay for carcinogenic or mutagenic effects would also be a desirable future inclusion in Tier IV.

Tier IV as described above corresponds to Tier IV in the Corps' comprehensive testing approach. This tier, the chronic/sublethal effects assessment, is usually not conducted at present because suitable tests for evaluating these effects are not sufficiently developed or standardized.

Ms. Coch emphasized that the tiered testing approach outlined above should not be considered the final answer to regulatory evaluation of PAH-contaminated dredged material, but only as a direction in which Corps Districts may proceed for the present. Considerably more research and information is needed to develop a detailed, comprehensive testing approach for PAH in sediment, particularly when chronic or sublethal effects are of concern. The Corps initially proposed progress in this direction under three work units of its Long-Term Effects of Dredging Operations (LEDO) Program, but due to funding cuts, only minimal efforts can presently be made under LEDO. The work units descriptions are included as Appendix D to this report. PAH-related research efforts currently proposed or in progress under the three LEDO work units include:

a. Methods development: determining the sensitivity of high-performance thin layer chromatography (HPTLC) for analyzing PAH in pure solution and in sediment (work unit 31772).

b. Use of radiolabeled fluoranthene to measure uptake by *Macoma* and empirically determine the bioaccumulation "preference factor" (i.e. magnitude of difference in steady state concentrations of a neutral organic chemical in organism lipid vs. sediment organic carbon (work unit 32571).

c. Compilation of a database on PAH residue-effects relationships from existing literature on PAH (work unit 31773).

The New York District has proposed a PAH study to be conducted under the Water Quality Research Program (Appendix E) for development of testing criteria for petroleum hydrocarbons in marine and freshwater. The ultimate goal of research under these programs is the development of scientifically sound, technically feasible regulatory guidance on PAH along with other contaminants of concern.

Test methods

A detailed description of the recommended tests or discussion of their methods was beyond the scope of the workshop. However, several questions were posed, either during the workshop or in the questionnaire, pertaining to general test methodology.

Test media. Question B.10 of the preworkshop questionnaire (Appendix C) asked in which specific phase (solid phase, water column, pore water) each biological test (recommended in response to Question B.9) should be conducted. All of the respondents, as well as the New York District, emphasized the importance of using solid phase tests for regulatory evaluation of dredged material. Four respondents also recommended water column or suspended phase tests (i.e., elutriate tests) for specific cases, such as "greasy" sediment or disposal sites where much dispersal will take place. However, other respondents indicated that elutriate tests have little value for assessing sediment toxicity because exposures in the water column are localized and transitory.

Dr. R. Lee recommended using pore water tests in addition to the other phases. Mr. Miller inquired whether there is an accepted procedure for extracting sediment pore water, and what problems might be involved. Centrifugation and squeezing are two methods of extraction. Dr. Spies said there is a concern about oxidizing the sediment unduly during the extraction, or altering physical-chemical properties by squeezing. He recommended using argon or nitrogen to drive the pore water out. Dr. Mac mentioned that using *in situ* pore water collectors is preferable to centrifugation from a geochemical standpoint, but that only very small amounts of water can be collected. This might be sufficient for pore water tests using microorganisms.

Other extracts of sediment, such as organic-solvent, aqueous or saline extracts, must be used for certain tests such as the Microtox test and the

Ames test. Dr. Petty identified some problems with using organic-solvent extracts. Such extracts are ostensibly collecting from the sediment more than what is biologically available, and thus do not approximate a real-world exposure. Furthermore, once the chemicals are removed from the sediment matrix, chemical reactions such as oxidation or coupling are more likely to occur. Such reactions can seriously alter the nature of the contaminants. Aqueous or saline extracts may be more representative of real-world exposures, but are poor at extracting lipophilic compounds such as PAH.

The participants agreed that using whole sediment (solid phase) exposures in the biological tests, if at all possible, is vastly preferable to using an extract or water fraction.

Amount of sediment for testing. Question B.7 of the preworkshop questionnaire (Appendix C) asked how much sediment would be needed for PAH testing in the tiered testing approach [proposed at the 1986 PAH workshop]. Responses ranged from a few grams to several kilograms for chemical analysis, and several kilograms for biological tests. The actual amounts would depend largely on the number and types of tests performed, and the size of the organisms used. Dr. Spies indicated that most sampling grabs and wide-diameter cores should provide sufficient sediment for testing.

Recommended analytical methodology. Question B.8 of the questionnaire (Appendix C) requested analytical methodology for sediment and tissue analysis that currently can be used by contract laboratories not having research-level capabilities. The respondents were divided over recommendations for analytical methodology, with some preferring high performance liquid chromatography (HPLC) and others preferring gas chromatography - mass spectrometry (GC-MS). This topic was not discussed during the workshop. Dr. Petty did indicate that GC-MS provides a more specific detection than HPLC, but there will be some ambiguities nevertheless. Details concerning the various methods and protocols can be found in the individual responses to Question B.8 in Appendix C.

Quality assurance/quality control (QA/QC). Although quality assurance and quality control were not discussed during the workshop, they represent crucial aspects of analytical or test methodology. These terms may be defined as follows (Rand and Petrocelli 1985):

> **Quality assurance (QA)** is a program organized and designed to provide accurate and precise results. Included are selection of proper technical methods, tests, or laboratory procedures; sample collection and preservation; selection of limits; evaluation of data; quality control; and qualifications and training of personnel.

Quality control (QC) refers to specific actions required to provide information for the quality assurance program. Included are standardizations, calibration, replicates, and control and check samples suitable for statistical estimates of confidence of the data.

Question B.12 of the questionnaire (Appendix C) asked for recommended QA/QC procedures for sediment analysis and biological testing. The respondents mentioned several QA protocols, including USEPA Methods, New Jersey Department of Environmental Protection procedures, National Oceanic and Atmospheric Administration (NOAA) Status and Trends guidelines, and the NOAA/NBS Program. Specific QC procedures listed for sediment analysis include performance evaluation materials, methods blanks, blird QC samples, spikes, matrix spike duplicates, check solutions, calibration standards, lab certification, standard extraction protocol, and internal standards. For biological testing, QA/QC procedures mentioned by the respondents include use of native sediments, a proper reference sediment, and a proper grainsize control. Laboratories performing bioassessments for the New York District are inspected by the Corps and USEPA, including their QA/QC programs. Protocols for these programs are specified in the Guidance Manuals used by the New York District (USEPA/CE 1977, CE/USEPA 1984).

PART III: SUMMARY OF MAJOR AGREEMENTS AND RECOMMENDATIONS

The following items may be considered points of general agreement arising from the workshop discussions and questionnaire responses:

a. The list of 15 priority pollutant PAH recommended at the 1986 PAH workshop for regulatory evaluation of petroleum hydrocarbons in dredged material should remain unchanged for the present. These 15 selected PAH are: acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo-[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, inden [1,2,3-cd]pyrene, phenanthrene, and pyrene.

b. Classes of hydrocarbons other than the priority pollutant PAH, such as the alkyl-, nitrogen-, and sulfur-substituted PAH, could have major toxicological importance but require more research before it can be determined whether representative compounds from these classes should be added to the list.

c. Numeric guidelines or thresholds for PAH in sediment or in tissues could serve as a screening tool for identifying sediments having sufficiently low (or high) PAH concentrations as not to require further testing. However, attempting to specify those threshold concentrations is not currently recommended because there are no "levels of concern" for PAH and little information as yet on which to base scientifically sound numeric guidelines.

d. Reliance on biological tests rather than on numeric guidelines for PAH in sediment is necessitated by current lack of understanding of the complex factors influencing bioavailability and toxicity. However, chemical analysis of sediment is nonetheless important for interpretation of contaminant tissue residues in organisms exposed to that sediment.

e. Chemical analysis of sediment is not sufficient to identify potential toxicity problems because toxicity may be caused by compounds present in the sediment but not included in the chemical analysis. Biological testing, at least for acute toxicity, is generally necessary in addition to the sediment analysis.

f. If acute toxicity tests were not included in the regulatory evaluation protocol, then it would be necessary to analyze the sediment for some of the more volatile (and acutely toxic) hydrocarbons, such as naphthalene, in addition to the 15 selected PAH.

g. Aquatic organisms that feed at the sediment surface or are deposit-feeders will have maximum exposure to sediment-associated PAH. Among these organisms, animals having well-developed metabolic capability for PAH, such as benthic fish and some crustaceans, will be most likely to experience toxicity.

h. Animals having limited ability to metabolize PAH, such as bivalve molluscs, will generally experience low acute toxicity due to PAH. However, they are good indicators of PAH bioavailability because they accumulate parent PAH compounds in their tissues. Bioavailability can be demonstrated using bioaccumulation tests.

i. Besides acute toxicity, PAH can cause adverse effects on growth and reproduction. PAH may also be linked to carcinogenicity or mutagenicity in susceptible organisms. Many of these adverse effects are probably caused by PAH metabolites rather than by the parent compounds. However, analysis of PAH metabolites is currently not technically feasible for regulatory evaluations.

j. Potential adverse affects of PAH on growth and reproduction can be assessed using whole or partial life cycle tests.

k. An adequate regulatory program for evaluating PAH-contaminated dredged material should incorporate a suite of laboratory tests or a combination of laboratory tests and field assessments. Arranging the tests in a tiered approach will enable the regulator to determine the number and progression of tests needed for a specific project evaluation.

m. The suggested tiered testing approach for PAH assessment includes three tiers that can be implemented now. These are an elaboration of the tiered scheme proposed at the 1986 PAH workshop, and correspond to Tiers I-III of the Corps' comprehensive testing strategy for aquatic disposal as part of the Federal Standard.

n. Tier I is the determination of "reason to believe" that the dredged material under consideration is contaminated with PAH, and that the potential exists for adverse biological effects to occur as a result of dredging and disposal operations. Tier II involves chemical analysis of the sediment for the 15 selected PAH. Tier III is the first biological testing tier and includes acute toxicity tests using sensitive organisms that are representative of organisms at the disposal site. Tier III also includes bioaccumulation tests using deposit-feeding organisms that have little metabolic capability for PAH.

o. Suggested organisms appropriate for Tier III acute toxicity tests include *Mysidopsis*, *Palaemonetes*, *Nereis*, and amphipods for saltwater; *Daphnia*, *Ceriodaphnia*, algae, larval fathead minnows, *Chironomus*, and *Hexagenia* larvae for freshwater.

p. Suggested organisms appropriate for Tier III bioaccumulation tests include *Macoma* and *Yoldia* for saltwater; *Pontoporeia* and *Hexagenia* for freshwater.

q. In both Tiers II and III, comparison should be made between dredged material and an appropriate reference sediment. If the dredged material test results are statistically significantly greater than the reference test results, then there is the potential for unacceptable adverse biological impacts to occur as a result of dredging and disposal operations. For the present, comparisons could be made using "total PAH" as the sum of the 15 individual PAH.

r. The two petroleum hydrocarbons workshops raised more questions about PAH than provided answers. Thus, the tiered testing approach outlined above should not be considered the final answer to regulatory evaluation of PAH-contaminated dredged material, but only as a direction in which Corps Districts may proceed for the present. Considerably more research and information are needed to develop a detailed, comprehensive testing approach for PAH in sediment. Research in this direction has been proposed by the Corps under the Long-Term Effects of Dredging and Water Quality Research Programs (Appendices D and E).

s. Recommendations for research and development include: compilation of a database relating environmental levels of the 15 PAH with biological effects; establishment of technically defensible, numeric regulatory criteria for PAH from this database; and development/standardization of sublethal effects tests (Tier IV of the Corps' comprehensive testing strategy).

t. Tier IV should include a partial or whole life cycle test for sublethal effects on reproduction and/or growth. A test for carcinogenic or mutagenic effects may also be desirable for future inclusion in this tier. Additional research and development will be necessary to provide standard laboratory analyses for these proposed bioassessments.

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Table 1

Suggested Bioassessment Organisms

Organism	Bioassessment	Test Medium	Comments
	Freshwater Organisms		
ALGAE			
Cladophora	Bioaccumulation	Water column	Uptake of metals but not organic chemicals
Selenastrum	Acute toxicity	Water column	
CRUSTACEANS			
Pontoporeia hoyi (amphipod)	Bioaccumulation Acute toxicity	Solid phase	Indigenous to Great Lakes, difficult to collect in winter, must be kept cold and in the dark, low PAH biotransformation ability
Hyalella (amphipod)	Growth/reproduction (partial life cycle)	Solid phase	
Daphnia pulex or D. magna	Acute toxicity Chronic (28-d) toxicity Reproduction (partial life cycle)	Water column or pore water	Short life cycle, easy to culture
Ceriodaphnia	Acute toxicity Reproduction	Water column	Native to Great Lakes, short life cycle, but difficult to culture in laboratory
INSECTS			
Chironomus tentans (midge)	Growth (10-d or 28-d) (partial life cycle) Acute toxicity	Solid phase	Native to Great Lakes, ASTM draft protocol available for 28-d growth test
Hexagenia (mayfly)	Acute toxicity		
FISHES			
Pimephales promelas (fathead minnow)	Acute toxicity Growth (partial life cycle)	Solid phase	
	Marine and Estuarine Organisms		
POLYCHAETE ANNELIDS			
Nereis virens (sandworm)	Bioaccumulation	Solid phase	Currently used by New York District
Dinophilus (archiannelid)	Mortality, growth, reproduction (whole life cycle)	Pore water	Short life cycle, sensitive to contaminants
BIVALVE MOLLUSCS			
Mercenaria mercenaria	Bioaccumulation	Solid phase	Currently used by New York District
Macoma	Bioaccumulation	Solid phase	Hardy deposit feeder
Yoldia	Bioaccumulation	Solid phase	Deposit feeder
CRUSTACEANS			
Palaemonetes pugio (grass shrimp)	Bioaccumulation Respiration rate	Solid phase	Currently used by New York District
Mysis relicta (mysid shrimp)	Bioaccumulation	Water column	Slight amount of PAH biotransformation
Rhepoxynius abronius (amphipod)	Growth/reproduction Acute toxicity	Solid phase	Moderate PAH biotransformation, may be sensitive to grain size
Eohaustorius (amphipod)		Solid phase	Little PAH biotransformation
Mysidopsis (mysid shrimp)	Acute toxicity Reproduction (partial life cycle)	Water column	Currently used by New York District, short life cycle
Ampelisca (amphipod)	Acute toxicity	Solid phase	Does not reproduce in laboratory

(Continued)

Table 1 (Concluded)

Organism	Bioassessment	Test Medium	Comments
FISHES			
Menidia menidia (silversides)	Growth/reproduction (partial life cycle)	Water column	
Citharicthys (sand dab)	Growth P-450 enzyme induction	Solid phase	West coast flatfish
Leiostomus xanthurus (spot)	P-450 enzyme induction		
Micropogonius undulatus (croaker)	Cataract development		
	Other Organisms		
BACTERIA	Microtox	Sediment extract	Reduction in bioluminescence as an indication of toxicity
	Ames	Sediment extract	Indicates potential mutagenicity
NEMATODES			
Pannogrellus	Acute toxicity Reproduction	Pore water	Short life cycle, sensitive to contaminants, easy to maintain in laboratory, microscopic
ANNELIDS			
Lumbricus terrestris (nightcrawler)	Bioaccumulation	Solid phase	Deposit feeder, survives in oxygenated sediment, ability to biotransform PAH ?
BIRDS			
Mallard eggs	Embryotoxicity	Sediment extract	

Table 2

Concentrations of PAH in Sediments or in Tissues from "Clean" Areas, Considered to be "No Effect" Levels, or Designated as Screening Level Concentrations, in Response to Question B.4 (Appendix C)

PAH Concentration	Description	Source of Information*
	Benz[a]anthracene	
0.52 ppm in sediment (2% TOC)	Screening level concentration	1
1.30 ppm in sediment (5% TOC)	Screening level concentration	1
	Benzo[a]pyrene	
0.001-0.005 ppm in sediment	"Clean" areas	2
0.79 ppm in sediment (2% TOC)	Screening level concentration	1
1.98 ppm in sediment (5% TOC)	Screening level concentration	1
0.03-0.05 ppm in sediment	Relatively clean areas around the Great Lakes	3
0.0005 ppm in mussels	Background levels	4
	Chrysene	
0.77 ppm in sediment (2% TOC)	Screening level concentration	1
1.92 ppm in sediment (5% TOC)	Screening level concentration	1
0.075 ppm in sediment	Relatively clean areas around the Great Lakes	3
	Fluoranthene	
0.86 ppm in sediment (2% TOC)	Screening level concentration	1
2.16 ppm in sediment (5% TOC)	Screening level concentration	1
0.08-0.10 ppm in sediment	Relatively clean areas around the Great Lakes	3
	Phenanthrene	
0.52 ppm in sediment (2% TOC)	Screening level concentration	1
1.30 ppm in sediment (5% TOC)	Screening level concentration	1
0.03-0.07 ppm in sediment	Relatively clean areas around the Great Lakes	3
	Pyrene	
0.87 ppm in sediment (2% TOC)	Screening level concentration	1
2.17 ppm in sediment (5% TOC)	Screening level concentration	1
0.05-0.10 ppm in sediment	Relatively clean areas around the Great Lakes	3
	Total PAH	
3.8 ppm in sediment	No effect level based on sediment quality triad	5
0.076 ppm in clams	Remote areas, Oregon	6
0.05-0.14 ppm in mussels	"Clean" areas	2
0.986 ppm in mussels	Remote areas, Oregon	7

* 1. R. Peddicord, 2. R. Lee, 3. M. Mac, USFWS data, 4. Dunn and Young 1976, 5. Chapman 1986, 6. Mix and Schaeffer 1983a, 7. Mix and Schaeffer 1983b.

Table 3

Concentrations of PAH in Sediments or in Tissues from "Contaminated" Areas, or Associated with "Major Biological Effects," in Response to Question B.5 (Appendix C)

PAH Concentration	Description	Source of Information*
	Acenaphthene	
2.5-7.5 ppm in sediment	Contaminated Great Lakes areas	1
	Acenaphthylene	
8-20 ppm in sediment	Contaminated Great Lakes areas	1
	Anthracene	
1-15 ppm in sediment	Contaminated Great Lakes areas	1
	Benz[a]anthracene	
2-25 ppm in sediment	Contaminated Great Lakes areas	1
	Benzo[a]pyrene	
2.5-20 ppm in sediment	Contaminated Great Lakes areas	1
10-15 ppm in sediment	Rivers passing through heavily populated or industrial areas	2
21 ppm in mussels	Contaminated areas	3
	Benzo[b]fluoranthene	
56 ppm in mussels	Contaminated areas	3
	Benzo[g,h,i]perylene	
1-15 ppm in sediment	Contaminated Great Lakes areas	1
	Chrysene	
3-30 ppm in sediment	Contaminated Great Lakes areas	1
	Fluoranthene	
7-35 ppm in sediment	Contaminated Great Lakes areas	1
3.3-10.5 ppm in sediment	Acutely toxic to *Rhepoxynius*	4
	Fluorene	
2-15 ppm in sediment	Contaminated Great Lakes areas	1
	Indeno[1,2,3-cd]pyrene	
1.5-15 ppm in sediment	Contaminated Great Lakes areas	1

(Continued)

* 1. M. Mac, USFWS data, 2. Neff 1979, 3. Bjørseth, Knutzen, and Seki 1979, 4. Swartz 1987, presented at 8th Annual meeting of Society of Environmental Toxicology and Chemistry, Pensacola, FL, 5. Chapman 1986, 6. P. Landrum, 7. R. Spies, 8. Mix and Schaeffer 1983a, 9. Mix and Schaeffer 1983b, and 10. R. Lee.

Table 3 (Concluded)

PAH Concentration	Description	Source of Information
	Phenanthrene	
5-100 ppm in sediment	Contaminated Great Lakes areas	1
	Pyrene	
5-50 ppm in sediment	Contaminated Great Lakes areas	1
	Total PAH	
6.8 ppm in sediment	"Major effects"	5
446 nmol/g in sediment	Toxic to *Pontoporeia hoyi*	6
5 ppm in sediment	Oakland area, California	7
0.585 ppm in clams	Bay front areas, Oregon	8
2.724 ppm in mussels	Bay front areas, Oregon	9
1-3 ppm in mussels	Heavily polluted areas	10

Table 4

General Biological Effects of PAH

in Different Groups of Aquatic Organisms

Organism	Biological Effect
Amphipods	Most likely to show toxicity
Molluscs	Low toxicity due to limited ability to metabolize PAH
	Good bioaccumulation
	Subcellular/cellular pathology
Invertebrates in general	Acute toxicity
	Bioaccumulation
	Chronic/reproductive effects
Fishes	MFO induction
	Reproductive effects
	Carcinogenicity

APPENDIX A: SCOPE OF WORK

Regulatory Interpretation of Petroleum Hydrocarbons in Dredged Material

for
US Army Engineer District, Chicago

Background

Concerns about possible environmental impacts of dredging and dredged material disposal are often based, at least in part, on the likely presence of petroleum hydrocarbons in the sediment. Regulatory analyses of dredged material and/or tissues of animals exposed to it have often included quantification of total oil and grease or total petroleum hydrocarbons in response to this concern. Scientific advances over the last several years have made this degree of analytical sophistation increasingly inadequate, either to accurately assess the potential for environmental impact or to allay concerns expressed by the public or other agencies. Literally hundreds of the individual compounds known collectively as petroleum hydrocarbons have been identified in sediment, water, and tissue samples. The complex variety of compounds that make up petroleum hydrocarbons spans a wide range of water solubility, persistence, bioavailability, toxicity, bioaccumulation potential, carcinogenicity, and overall biological importance. The environmental significance of any specific sample is determined by the particular mix of compounds that make it up. For this reason "summary" type analyses, such as total oil and grease or total petroleum hydrocarbons, cannot provide sufficient information to accurately evaluate the potential for environmental impact of petroleum-contaminated samples. Two samples with the same total petroleum hydrocarbon content can often be of vastly different environmental concern when one consists largely of compounds of relatively low bioavailability, persistence, toxicity, and overall biological importance, and the other has important quantities of bioavailable, persistent, toxic, bioaccumulative, and/or carcinogenic compounds.

Clearly the summary type analyses are inadequate for regulatory purposes, and more precise and interpretable analyses are needed. However, it is equally clear that exhaustive analyses of all petroleum compounds present would be far too time consuming and costly and would produce an unwieldy volume of data for regulatory purposes. What is needed is to simplify the complexity that is petroleum hydrocarbons by focusing on clearly identified key compounds, or classes of compounds, that are of the most importance environmentally. In this manner, adequate resolution for defensible evaluations could be obtained at a time and cost that are practical in the dredged material regulatory program.

The public, state, and other Federal agencies are placing increasing emphasis on petroleum hydrocarbon evaluations. Not all these activities are scientifically sound, and most do not consider the economic and administrative factors important to the Corps of Engineers' regulation of dredged material. The Corps' interest and public image would be well served by development of a technically sound and practically implementable approach to regulatory evaluation of petroleum hydrocarbons in dredged material.

In a letter of 8 May 85 to the attention of Dr. Richard Peddicord at the WES, the Chicago District's Commander and Director requested assistance to Mr. Jan Miller in advancing the technical approach to regulatory evaluation of petroleum hydrocarbons in dredged material. Need for assistance was identified in the following general areas: [a] identifying a manageable number of key components of the petroleum hydrocarbon mixture that are most appropriate for regulatory purposes, [b] development of guidance on environmental evaluation of particular levels of these components in sediments that may be dredged, and [c] assessment of dredging and disposal in Great Lakes harbors in light of [a] and [b].

Objectives

The proposed work will address the first two of the aforementioned areas of interest and will provide [a] identification of the particular components of the complex petroleum mixture that are most appropriate for analysis as a basis for regulatory evaluation of sediments proposed for dredging, and [b] guidance on the state-of-the-practice scientific interpretation of potential environmental impacts of the petroleum hydrocarbon components identified in objective [a].

Approach

Past experience has proven that the most productive way to arrive at consensus findings in complex scientific areas is through a technical working group of experts. Therefore, a group of 8 to 12 widely recognized authorities with extensive expertise in environmental impacts of petroleum hydrocarbons in sediments will be identified. Those selected will be carefully chosen to include scientists from government, academia, and the private sector who have knowledge of dredging, disposal, and the dredged material regulatory process. A representative of the Chicago District will be included, and the District will be consulted in the identification of other participants. Those chosen will be provided a statement of goals and objectives, and will be asked to produce a written description of their perceptions and suggestions and to be prepared to elaborate and justify their inputs at the workshop. This premeeting work will help form the basis of the final agenda as well as maximize the amount of valuable workshop time that can be devoted to productive interactive discussion. At the conclusion of the workshop, the WES will then prepare a report in the form of a WES Miscellaneous Paper summarizing the working group goals, activities, conclusions, and recommendations. The report will be supported not only by the expertise of the participants, but also by justification provided by participants from the scientific literature for specific conclusions.

The USAED, New York (NYD), also contacted the WES seeking technical assistance in similar areas concerning regulatory evaluation of petroleum hydrocarbons in sediment. Because of the similarities in the two requests, complementary responses were prepared. A separate Scope of Work was submitted to NYD for funding to accomplish the first objective stated above for this Chicago work. A workshop was conducted for the NYD during FY 86 and a report of the workshop proceedings was prepared. Thus, the first of the Chicago District's objectives as stated above was accomplished by the NYD-sponsored work, and the Chicago District benefitted through attendance at the workshop and receipt of the report. We propose that the second objective be met by the

work sponsored by the Chicago District, and that the NYD likewise benefit from this work. Each District would have paid for one Scope and would have its objectives fully met by receiving full benefit of both efforts. This provides an unusual opportunity for very timely and cost-effective mutual benefit on an important environmental matter.

Product

A report will be prepared describing the study objective, methods, findings, and conclusions. Conclusions will be supported on the basis of the consensus of the recognized authorities participating in the workshop, and selected documentation from the scientific literature. A complete draft report will be submitted to the Chicago District for review and comment prior to preparation of the final report. The final report will be published as a WES Miscellaneous Paper. Sufficient copies will be published for limited distribution of WES reports, as well as 100 copies to be supplied to the Chicago District.

Schedule

	Event	Accomplished by
1.	Agreement on scope and receipt of funding by the WES	30 Sep 87
2.	Selection of participants in conjunction with Chicago District	1 Dec 87
3.	Receipt of preworkshop input from each participant	1 Mar 88
4.	Finalize commitment for participants to attend meeting	1 Mar 88
5.	Workshop conducted at WES	15 Mar 88
6.	Draft report to Chicago District and participants for review	1 Jul 88
7.	Comments from reviewers received at WES	15 Aug 88
8.	Final report to Chicago District for approval for publication	2 months after event 7 is accomplished
9.	Final approval from Chicago District for publication received at WES	1 month after event 8 is accomplished
10.	Published report distributed	6 months after event 9 is accomplished

APPENDIX B: STATEMENTS OF OBJECTIVES

Chicago District Goals For Workshop on Polynuclear Aromatic Hydrocarbon (PAH) Compounds in Dredged Material

December 1987

1. Background

1.1 The Chicago District is responsible for regulating the disposal of dredged material from portions of the Great Lakes and other inland waterways through Section 404 of the Clean Water Act (CWA). In addition, the District also dredges bottom sediments from a number of Federal navigation projects. Dredged material disposal criteria for the Great Lakes were established by USEPA Region V under Section 404 (b) of the CWA. These criteria are based on bulk chemical concentrations of the dredged material.

1.2 Polynuclear aromatic hydrocarbons (PAHs) are present in the bottom sediments of many harbors and waterways around the Great Lakes. Presently there are no dredged material disposal criteria or guidance in the Great Lakes concerning PAH contamination. In order to provide for a reasoned, scientific approach to the regulation of PAHs in dredged material, the Chicago District and New York District have sponsored workshops on this subject at the U.S. Army Engineer Waterways Experiment Station (WES).

2. Goals and Objectives

2.1 The ultimate goal is to have a scientifically valid procedure for decisionmaking in regard to the regulation of dredged material which may contain PAHs. To do this, the Corps must have both tools and criteria. Tools are the testing procedures used to establish the level or concentration of the parameter(s) on which criteria are based. This workshop should first consider the type(s) of criteria which can be employed, and then recommend the most appropriate tools to measure with.

2.2 Regulatory decisions on dredged material disposal are best conducted using a tiered approach. For the Great Lakes, bulk chemistry has been a large part of this process and is logically the first tool to be employed. Sediments which have PAHs below some level of concern (all other pollutants being absent) could be considered acceptable for open-water disposal. Sediments with gross levels of PAH contamination would never be considered for unrestricted, open-water disposal. Sediments with detectable levels of PAHs, but less than "gross" levels, might be acceptable for open-water disposal based on additional testing.

2.3 The first criteria must define what concentrations of PAHs in sediments are below a level of concern and what levels are considered "gross." Sediments with concentrations below the first or above the latter would require no further testing. These threshold concentrations are only a secondary goal of this workshop. Participants should consider and discuss this concept and bring any information with them which might define these lower or upper limits.

2.4 The primary goal of the workshop pertains to the tools and criteria relevant to dredged material which fall between these two extremes. Sediments having levels of PAHs above some lower level of concern, but not so high that open-water disposal is completely unthinkable. For this dredged material, criteria will most probably be based on bioassay/toxicity tools.

2.5 The workshop participants should discuss pertinent biological testing protocols which would be applicable to dredged material with PAHs. The end-products should be a recommended suite of tests for evaluating the impacts of open-water disposal of these materials. This should include no more than four (4) testing procedures, with a discussion of the advantages and limitations of each.

2.6 A detailed discussion of criteria based on these biological testing procedures is beyond the time constraints of this workshop. However, participants should bring to the workshop any information on "action-levels" for PAHs or studies relating PAH induced impacts which would contribute to the District's general knowledge on the subject.

Jan A. Miller
Environmental Engineer

New York District (CENAN) Objectives for Second
Petroleum Hydrocarbon Workshop (March 1988)
CENAN Objectives for Petroleum Hydrocarbons
Workshop No. II

December 1987

. Build on the results of the previous PAH Workshop to recommend evaluative bioassay/bioaccumulation tests for priority pollutant PAH which cause adverse biological effects.

. The test should address the bioavailability and adverse impacts to biota: specifically in a 10-day bioassay/bioaccumulation assessment. Organisms used should be the same as those currently used by New York District for such tests, unless specific compelling reasons require additional species.

. Recommendations from the first Petroleum Hydrocarbons Workshop should be used as a starting point for discussion in the second workshop. The evaluative testing proposals should be acceptable to both New York District and Chicago District.

. Fifteen priority pollutant PAH's were identified for further study and a tiered testing scheme was proposed in the previous workshop. These recommendations should be used as a starting point for further discussion. New York District is primarily interested in developing procedures which will show the extent of bioavailability of PAH's from sediments, and its environmental significance. Bulk sediment analysis need not be performed for New York District analyses (bioassays and bioaccumulation tests will be used instead).

. All participants in the second PAH Workshop should be prepared to contribute towards gathering all available information on concentration and range of impacts on animals for the 15 PAH's.

. The participants will prepare list of organisms and how they are affected by the 15 PAH's and the range of concentrations which cause these effects both for individual PAH's and for the 15 combined PAH's.

. Questions which should be discussed in detail at the upcoming workshop include:

- What levels of PAH are significant in tissues?
- How does that vary depending on the species?
- What do these levels mean to the organism in terms of adverse effect?
- Can the matrix approach (using comparison with ambient values in a relatively "clean" area of the harbor) be used?

Carol A. Coch

APPENDIX C: COMPILED RESPONSES TO PREWORKSHOP QUESTIONNAIRE

A. Recommendations of the 1986 PAH Workshop

Question A.1. Do you feel strongly that any compounds should be added to or deleted from the list? If so, please state your justifications.

R. Lee. I have no additions or deletions to the PAH list.

R. Peddicord. The list of 15 priority pollutant PAHs agreed upon by the first workshop is a good place to start. Compounds can be added or deleted in the future as experience in the dredged material regulatory program warrants. I foresee a continuing refinement over the next several years of the approach to evaluating dredged material for potential environmental impacts associated with PAHs.

J. Petty. I believe the list of PAHs should remain the same. For the most part maintaining the priority pollutant PAH list adds to the perception of adequacy.

M. Mac. A number of other PAH compounds routinely appear in Great Lakes sediments and biota such as benzo[e]pyrene, 1-methylnaphthalene and dibenzothiophene (see more comprehensive list in Table C-1)[1]. In addition, nitrogen-containing PAH compounds are not well represented in Table C-1 although they are present in contaminated Great Lakes sediments and show genotoxicity. The problem arises when a limited number of compounds must be selected from an extremely long list of PAHs of which we know little about. Picking a representative list of compounds will always have some shortcomings.

P. Landrum. While the participants of the previous workshop considered that the aliphatic component of petroleum was not as important as the aromatic portion in terms of toxicity it could result in habitat alteration. There were several additional classes of compounds which the group considered potentially important but which did not have either a strong information base or an established routine analytical method, such as the nitrogen and sulfur containing aromatics. The absence of sufficient information to permit regulation was not to imply that these compound classes were harmless or that the polycyclic aromatics were the only xenobiotic class to be considered.

D. Stainken. I have reviewed the list of 15 key PAHs and the Summary of Major Agreements from the first PAH Workshop (Clarke and Gibson 1987a)[2]. In general, I concur that many of the listed compounds are indicators of PAH in the NY-NJ estuarine and marine areas. In earlier sediment analyses we identified the presence of benz-a-pyrene, anthracene, benz-anthracene, fluorescent profiles of numerous 3, 4, 5 and 6 ring PAH compounds as well as the fluorescent

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1. It should be emphasized that of the compounds listed in Table C-1, only those asterisked are currently recommended for inclusion in regulatory evaluations of dredged material.

2. References cited in this Appendix can be found in the References section of the report.

profiles of naphthalenes, methyl substituted naphthalenes and pyrenes. These sediments were sampled throughout the lower NY Bay-Raritan Bay Complex (Stainken and Frank 1979, Stainken 1979). In other work (Stainken 1983), almost all of the listed PAH have been analyzed from sediments of the NY Bight. These analyses also found naphthalenes and methyl and dimethyl naphthalenes to be prevalent as well as dibenzothiophene.

Based on our findings, I disagree with not including the naphthalenes and methyl naphthalenes on this list. It has been my experience (Stainken and Frank 1979; Stainken 1975, 1977, 1979, 1983) that the lower molecular weight methylated naphthalenes, xylenes (single ring) and phenanthrene tend to bioaccumulate and do occur in sediments. The list should clarify that the methyl isomers are to be quantitated, particularly dimethylnaphthalene. Much of our research indicated the consistent occurrence of methylated naphthalene in harvested clams and oysters from NY Harbor, and in experimental studies. A comparison of PAHs in six species of bivalves relative to the overall distribution of aromatics in Raritan Bay indicated that lower molecular weight PAHs (i.e. 1, 2 and 3 ring compounds and methyl isomers) selectively accumulate in bivalves. In the first workshop, issues of volatility were raised and concern was expressed that the naphthalene class rapidly volatilized. However, our experience has been that the yearly average bottom temperatures generally ranged from 4-12°C and volatilization is not necessarily a major factor under normal estuarine and marine temperature regimes.

J. Stein. The list of PAHs generated during the first workshop is sufficiently representative of PAHs as a class of chemicals.

C. Rice. No, I see no reason to alter the list of 15 PAHs which you have selected.

R. Spies. The fifteen PAH listed appear to be a reasonable starting point based on our current knowledge of contaminant occurrence and effects in the aquatic environment. However, our knowledge of the importance of other groups of aromatic compounds (e.g., azoarenes, aromatic amines and alkyl-substituted PAH) is poorly developed and it should be recognized that not all compounds of major toxicological importance have been identified.

Question A.2. Are there any groups of compounds that have shown parallel changes in concentration, possibly indicating a common source and the need to analyze for only one of them?

R. Lee. Many of these PAH compounds could come from a common source, but it is difficult to recommend deletion since so many were examined before arriving at the 15.

R. Peddicord. This issue should be re-examined when a couple of years data on dredged material from around the country are available; a decision now would be speculative.

J. Petty. Not that I am aware of.

M. Mac. No.

P. Landrum. Groups of compounds, such as the PCB and toxaphene, that have been studied in many environmental scenarios have pointed out the need to fol-

low all the components individually. This becomes more important as our understanding of the disposition and processes acting on the compounds become better understood.

D. Stainken. In the NY Harbor area, there appears to be a generalized trend of an increased amount of PCB and total extractable hydrocarbons relative to total amounts of PAH present (Stainken and Rollwagen 1979; Stainken, Multer and Muicki 1983; Stainken 1984). There did not appear to be a common source for specific compounds. Sampling surveys indicated that most materials derived from intense vessel traffic and sewage discharge. Because of the recent development of the presence of dioxin materials in upper bay sediments, it would be prudent to analyze for their presence as a class (i.e. dibenzofurans and dioxin).

J. Stein. Most analytical methods for PAHs provide information on the 15 key PAHs as well as for many other PAHs. There would be little cost savings by analyzing for fewer compounds.

C. Rice. No, I am not aware of any group of compounds showing parallel changes in concentration in the environment which would allow only one of them to be monitored.

R. Spies. To answer this question I have analyzed our data on PAH in about 40 samples of San Francisco Bay sediment to determine the pairwise correlation between 13 PAH (most of those on our list). The resulting correlation matrix of TOC-normalized concentrations in this sample set is attached (Table C-2). Pearson's r is generally above 0.7 for these comparisons and the mean is considerably higher. So it appears that, in general, measurements of a few of these compounds will provide the data to reasonably predict the rest. I would suspect that other estuaries with mixed inputs might also show such a pattern. However, since most of the effort and cost has to be spent anyway to obtain a few good measurements, the remaining compounds are obtained at little extra cost, i.e., the costs are not linearly related to the number of compounds analyzed.

Question A.3. Can any of the PAHs be used as target compounds for specific environments or incidents (e.g. runoff, leaching, creosote, oil spill)? What is the most likely source for each of the 15 PAHs in the aquatic environment, particularly New York Harbor and the nearshore areas of the Great Lakes?

R. Lee. Most of the PAH on the list would be by-products of fossil fuel combustion, e.g. diesel exhaust. The alkylated PAH are often in relatively high abundance in crude oil, but these alkylated PAH are not on the list. I would predict that the source of the 15 PAH in the aquatic environment would include the following: (1) deposition of air particulates containing combustion by-products (particularly high molecular weight PAH such as benzo[a]pyrene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene); (2) crude or refined oil could be the source of lower weight PAH, such as fluorene, fluoranthene and phenanthrene; (3) combustion by-products produced in the water would include both low and high molecular weight PAH.

Wakeham (1977) in a study in Lake Washington has suggested that PAH containing 5 or more rings, e.g. benzo[a]pyrene, come primarily from the atmosphere through the combustion of fossil fuels, since stormwaters did not have high weight PAH.

J. Petty. Because the priority pollutant PAHs are common to many sources (e.g. petroleum, coking, coal, etc.) it would be difficult to fingerprint sources solely on the occurrence of these contaminants in the sediment samples. The most likely sources of these materials are anthropogenic in nature.

M. Mac. According to Eadie (1984) atmospheric input appears to be the major source of PAH to the open waters of the Great Lakes. The highest concentrations found in nearshore areas have been associated with the coking facilities. Other industries that emit PAH include aluminum smelting. A number of fuel combustion sources provide PAH contaminants. Data on PAH levels in various effluents, oils, and sludges can be found in a National Research Council Canada (NRCC) report on polycyclic aromatic hydrocarbons in the aquatic environment (NRCC 1983).

P. Landrum. The PAH can be segregated somewhat into combustion-related compounds versus oil components. Petroleum and low temperature combustion sources tend to contain more of the alkylated PAH while high temperature combustion tend to be more the parent unalkylated congeners. Ratios of specific congeners can be used in some cases to distinguish sources. In the Great Lakes the sources of PAH include coking for iron and steel manufacture, coal combustion, home heating with wood, and other combustion sources as well as urban runoff and occasional petroleum spills. Some of the most polluted sediments in the Great Lakes are a result of the coking operation (Eadie 1988).

D. Stainken. All of the PAHs listed can be used as target compounds to indicate the presence of crude and refined oils, waste oils, creosote, etc. (Zafiriou et al. 1977; Frank, Stainken and Gruenfeld 1979; Tanecredi and Stainken 1981). An ASTM PAH fingerprint technique exists using fluorescence spectroscopy as a means of identifying specific oils and contaminant sources. Generally, some type of GC profiling is also needed for interpretation and quantitation. It is doubtful if the 15 PAHs can be used specifically as indicators of runoff or leaching without comparative sampling of suspect sources.

J. Stein. The use of single PAHs or a small suite of PAHs as indicators of specific sources is generally not appropriate. Moreover, it would be extremely difficult to identify the source of individual PAHs. A complete profile and composition or "fingerprint" of the PAHs present in sediment from a site is needed to identify possible sources. The major sources of PAHs appear to be from pyrolysis of petroleum by way of non-point source discharges. However, there are other sources that can be very important at specific sites, such as waters near creosote operations, for example.

R. Spies. We have identified several heteroaromatic compounds, benzthiazoles, that appear to be good indicators of street runoff (Spies, Andresen and Rice 1987). Some of these compounds appear to be persistent in estuarine sediments and are derived from antioxidants used in tire manufacture. The concentrations of these compounds may eventually provide information on the amount of PAH due to street runoff..

B. Sediment Analyses and Biological Testing

Question B.4. Can you specify for any of the 15 PAHs a "level of concern" in sediments or in tissues, below which that compound is unlikely to have any adverse biological effects?

R. Lee. A number of studies have surveyed bivalves and sediments for PAH. Mix and Schaeffer (1983a) found total PAH concentrations in clams (*Mya arenaria*) in bay front areas of Oregon to average 585 ng/g while clams from more remote areas averaged 76 ng/g. For the mussel, *Mytilus edulis*, total PAH con centrations in bay front and remote sites were 2724 and 986 ng/g, respectively (Mix and Schaeffer 1983b).

Background levels of benzo[a]pyrene in mussels appear to be 0.5 ng/g or lower (Dunn and Young 1976). Thus concentrations higher than this are of concern. Mussels collected from contaminated areas contain levels of benzo[a]-pyrene and benzo[b]fluoranthene as high as 21 and 56 μg/g, respectively (Bjørseth, Knutzen and Seki 1979).

Total PAH concentrations in mussels from "clean" areas range from 50-140 ng/g while mussels from heavily polluted areas have PAH ranges from 1000-3000 ng/g. Lower weight PAH such as acenaphthene, acenaphthylene and phenanthrene are usually at low levels in mussels, even though these compounds may be at high concentrations in the sediments of the collecting site. This is probably due to rapid elimination of low molecular weight PAH.

Sediments in rivers going through heavily populated or industrial areas contain benzo[a]pyrene at concentrations as high as 10-15 μg/g (Neff, 1979). Sediments from "cleaner" areas are in the 1-5 ng/g range. High concentrations of benzo[a]pyrene are often reported near sewer outfalls. Thus, for benzo[a]pyrene, concentrations in the μg/g range should be of concern.

While it is clear that sediment PAH are bioavailable (Landrum et al. 1985, McCain et al. 1978, Tatem 1986, Varanasi et al. 1985) not enough studies have been carried out to relate sediment concentrations with tissue concentrations. Also, in the case of fish and many crustaceans, tissue concentrations may not reflect the amount of PAH taken up since these animals can carry out extensive PAH metabolism.

R. Peddicord. This question is being addressed in a number of ways by different groups. EPA's Criteria and Standards Division is developing sediment quality criteria by calculating partitioning from sediments to interstitial water and using water quality criteria as the indicator of acceptable concentrations in the interstitial water. Momentarily discounting technical reservations on the part of some scientists about the approach, it will be of little use for the 15 selected compounds until water quality criteria are developed for more of them.

Another way I consider potentially useful to determine "levels of concern" for contaminants in sediments is the screening level concentration (SLC) approach (Neff et al. 1987). The SLC is the sediment contaminant concentration in which a diverse benthic population can be shown to exist. Contaminant concentrations above the SLC are considered to have a potential to impact the benthic community and would warrant further investigation. For non-polar organic contaminants SLCs are expressed on an organic carbon-normalized basis,

and can be calculated from existing databases. The following values have been calculated for saltwater ecosystems for some of the PAHs on the list:

Compound	SLC μg PAH/g TOC	SLC (μg/g) 2% TOC	SLC (μg/g) 5% TOC
phenanthrene	25.9	0.52	1.30
fluoranthene	43.2	0.86	2.16
benz[a]anthracene	26.1	0.52	1.30
chrysene	38.4	0.77	1.92
pyrene	43.4	0.87	2.17
benzo[a]pyrene	39.6	0.79	1.98

A possible approach to consider for evaluating tissue concentrations might go something like the following. If the chronic water quality criteria are acceptable for aquatic life, the body burden that results from living in water at the water quality criteria is an equally acceptable body burden. If true, the chronic water quality criteria times a bioconcentration factor would give an acceptable tissue concentration. Even if this logic is acceptable, there are few chronic water quality criteria for the 15 compounds.

J. Petty. No. The nature of the interactions between sediment "bound" PAH and target organisms is extremely complex. I question the validity of extrapolations from limited data.

M. Mac. While the "no adverse biological effect" has not been demonstrated, the following levels have been found in relatively clean areas around the Great Lakes (USFWS data in ng/g, dry wt):

phenanthrene	30-70	fluoranthene	80-100
pyrene	50-100	benzo[a]pyrene	30-50
chrysene	75		

P. Landrum. In my search of the literature for information relating to the level of PAH in sediments that will result in an effect, the data are sparse. In recent studies in this laboratory, we found that for total PAH 466 nmol/g dry sediment (1% organic carbon content) was a 26-d LC50 for the Great Lakes amphipod, *Pontoporeia hoyi*. Dr. Richard Swartz reported that fluoranthene was acutely toxic (10-d LC50) to the marine amphipod *Rhepoxynius abronius* at 3.3 to 10.5 mg/kg sediment over an organic carbon content in the dry sediment of 0.2 to 0.5% (presented at the 8th Annual meeting of the Society of Environmental Toxicology and Chemistry, Pensacola, FL, November 1987). Using a triad approach, Chapman (1986) reported that the no effect level for total PAH in the presence of PCB and other organic and inorganic compounds for a marine environment was 3.8 μg/g sediment and that major effects could be found at 6.8 μg/g. The work by Chapman was for chronic effects in a real world situation.

T. Dillon. Identifying specific levels of "concern" or "gross contamination" has always been very difficult if not impossible. The problem is associated with two factors: (1) uncertainty associated with predicting potential impact; (2) chemical by chemical regulation of a <u>complex</u> mixture and all that implies. A more productive approach may be to evaluate <u>relative</u> differences rather than <u>absolute</u> numbers. This can be achieved by comparing sediment concentrations in project sediment to those at the disposal site and a reference area.

D. Stainken. Outside of literature values, we have not determined PAH concentrations of "concern" in sediments or tissues. Earlier toxicity tests (Stainken 1976, 1977) indicated that aquaria exposures of 10 ppm of fuel and crude oils could cause histopathologic effects and depletion of glycogen in bivalves. Although the actual concentration of PAH was unknown, the aromatic portion of the oil used was 14% or 1.4 ppm (mg/L). In studies (Stainken 1976) of the accumulation and depuration of #2 fuel oil, the effects of oil exposure were noticeable after 4 weeks exposure to 100 ppm of #2 fuel oil emulsion. The actual dose was 100 ppm initially but was measured weekly during 4 weeks to be 1.52 to 0.46 ppm of total hydrocarbon in the water column. Since much of the aqueous material was alkyl benzenes, di- and trimethyl naphthalenes, it may be deduced that these values may be harmful. If a safety factor were to be calculated, assuming an average exposure concentration of 0.77 ppm of aqueous PAH (based on an average experimental exposure during 4 weeks) reducing the chronic exposure by 10X would yield a concentration of 0.077 or 0.08 ppm of PAH as an assumed No Effect Level (NOEL).

J. Stein. Development of sediment quality criteria is currently receiving a great deal of attention, and is in the early stages of development. At present, there exists no meaningful estimates of apparent effects thresholds for individual PAHs below which there would be no adverse biological effects. Again, basing action levels on chemical analyses of sediment assumes we currently have a good understanding of the factors influencing the bioavailability of compounds, mechanisms of toxicity (acute or chronic) in species of concern, and effects of factors such as temperature and salinity, for example, on toxicity (Varanasi, Stein and Nishimoto, in press; Moore, Livingstone and Widdows, in press). Further it must be assumed that the environmental significance with regard to biological effects of one class of compounds can accurately be assessed without consideration of interactive effects between the many chemicals that can potentially be present at a contaminated site. In many of these areas we clearly lack a complete understanding, and thus, at present, must rely mainly on biological tests rather than numeric chemical data for identifying sediments of concern.

R. Spies. A rigorous answer to this question is not possible until the appropriate experiments have been carried out. However, some educated guesses are possible. Consider the following:

A. Two extensive reviews of the effects of oil pollution indicate that the apparent threshold for effects in the laboratory (Capuzzo 1987) and the field (Spies 1987) are in the area of 10-20 ppb (total dissolved hydrocarbons). I'm uncertain as to what equilibrium concentration values for total PAH in sediments these would correspond to, but these values might be available from the Marine Ecosystems Research Laboratory (MERL) reports from the University of Rhode Island. Also, these threshold effects are almost certainly due in part to mono and di-aromatic compounds, which are not among the 15 PAH decided on in the first workshop.

B. Extensive recent studies of the effects of contaminated sediments have been carried out on marine invertebrates and fishes in San Francisco Bay. Five stations were sampled, representing a gradient of urban contamination. The results of several tests (including sediment bioassays) and measurements indicated generally that the most contaminated area, Oakland, had sediments eliciting assay responses, although one test, occurrence of micronuclei in circulating erthrocytes of starry flounder, showed a bay-wide response rela-

tive to a coastal reference site. Sediments in the Oakland area have concentrations of total PAH generally above 5 ppm (dry).

C. In spite of the reservations I have about the triad approach (e.g., Chapman, Dexter and Long 1987) due to covariance of the several endpoints (organic contaminants, benthic community changes and potential sensitivity of assay organisms to fine grained sediments) with total organic carbon, these results have pointed towards effects occurring at thresholds of greater than several ppm (dry) total PAH. Again, the interpretation of such data for regulating PAH would have to be done cautiously because of the probable contribution of other compounds to toxicity.

While educated guesses must be considered tentative, such guesses may provide guidance to regulators on an interim basis that would be preferable to no guidance. Dumping will occur whether we have sufficient scientific knowledge about its effects or not. Hopefully, as the data to solve this problem accumulates over the next 5-10 years, we can refine our estimates of thresholds for PAH-induced damage in aquatic communities.

Question B.5. Can you specify for any of the 15 PAHs a threshold level of "gross" contamination in sediments or in tissues, above which that compound will most likely have unacceptable adverse biological effects?

R. Lee. (See response to Question B.4).

R. Peddicord. EPA views the sediment quality criteria mentioned in Question 4 as useful in this context. Their approach considers a sediment whose interstitial water exceeds the water quality criterion to pose an unacceptable threat of impact. Sediments whose interstitial waters are below the criteria are not considered on that basis alone since interactive effects of multiple contaminants are not known.

Rick Swartz of EPA has developed some data on toxicity of sediment-sorbed fluoranthene to amphipods. This work was presented at the 8th annual meeting of the Society for Environmental Toxicology and Chemistry in Pensacola, Florida in November, 1987. Rick is preparing a paper on this work for submission to the journal Environmental Toxicology and Chemistry. Rick studied the toxicity of a range of concentrations of fluoranthene in sediments to the amphipod *Rhepoxynius abronius*. He found that the concentration lethal in 4 days to half the test organisms was related to the organic carbon content of the sediment, and could be described by the equation:

$$C_s = -1.26 + 24.5(OC)$$

where C_s is the concentration of fluoranthene in mg/kg dry weight lethal to half the test animals, and OC is the organic carbon concentration in the sediment in g/kg dry weight. That is, in sediments of about 0.5% organic carbon, half the animals were killed in 96 hours at about 11 ppm fluoranthene.

J. Petty. No, though I am certain such data exist.

M. Mac. PAH concentrations in sediments from contaminated Great Lakes areas where PAH compounds are suspected of causing tumors in fish (USFWS data in ng/g, dry wt):

phenanthrene	5,000 - 100,000
fluoranthene	7,000 - 35,000
pyrene	5,000 - 50,000
benzo[a]pyrene	2,500 - 20,000
chrysene	3,000 - 30,000
acenaphthene	2,500 - 7,500
acenaphthylene	8,000 - 20,000
anthracene	1,000 - 15,000
benz[a]anthracene	2,000 - 25,000
benzo[g,h,i]perylene	1,000 - 15,000
fluorene	2,000 - 15,000
indeno[1,2,3-cd]pyrene	1,500 - 15,000

P. Landrum. (See response to Question B.4).

T. Dillon. (See response to Question B.4).

D. Stainken. Our field research in Raritan Bay did not differentiate many of the specific 15 PAHs. Our research indicated the following:

Analysis of sediment sample extracts has yielded measurable quantities of identifiable 1 to 6 ring aromatics with isomeric methyl-substituted naphthalenes the most prevalent. The range of PAHs in the water samples (5-20 ng/L) was substantially less than the mean values of PAHs contained in the bivalves (387 ng/g) and both the sub-littoral (911 ng/g) and intertidal (1172 ng/g) sediments. The abundance and relative distribution of PAHs reflected the biological, chemical and physical mechanisms of transport and accumulation within the estuary. The limited solubility of PAHs in the water column and their preferential adsorption to very fine sand and silt-clay enhances the deposition and retention of aromatic hydrocarbons in the sediment matrix. The primary circulation patterns within the Bay (i.e. a counter-clockwise gyre) facilitated the accumulation of PAHs (especially 3, 4, 5 and 6 ring compounds) in the sub-littoral sediments along the southern shore of Staten Island. Comparison of the PAHs in six species of bivalves relative to the overall distribution of aromatics in the bay indicated that the low molecular weight PAHs (i.e. 1, 2 and 3 ring compounds and their methyl isomers) selectively accumulate in the bivalves.

In a subsequent paper (Stainken 1984) several conclusions concerning the abundance of macrobenthos vs. sediment silt/clay, PAH and hydrocarbon contamination were shown.

a. There was a lack of correlation between diversity and specific pollutants.

b. As the sediment increases beyond 20% silt-clay, there is a continual decrease in numbers of species present.

c. As total organics (hydrocarbons including PAH, PCB and total petroleum) exceeds 300-400 ppm, the numbers of species present noticeably drops.

J. Stein. At present, a level of "gross contamination in sediment" cannot be precisely specified for any PAH or for that matter any sediment-associated compound above which the compound would have a deleterious biological effect. It should be noted that in studies of the effect of petroleum hydrocarbons on the "scope for growth" of the mussel (*Mytilus edulis*), no apparent threshold

level of effect was observed, when assessed over a wide range of tissue concentrations (Moore et al., in press).

R. Spies. (See response to Question B.4).

Question B.6. Do any type of defined regulatory criteria exist for any of the 15 PAHs?

R. Peddicord. I am not aware of defined regulatory criteria for any of the 15 compounds. Water quality criteria or advisories are under development for a few of them. The US Food and Drug Administration (FDA) has no limits for seafood for these compounds, but some other countries have FDA-type limits for some of them. Criteria might be developed based on acceptable daily intake levels and average seafood consumption rates.

M. Mac. There may be water or sediment quality standards for PAHs in some Great Lakes states of which I am unaware. Water quality criteria recommended for the protection of aquatic life in the Great Lakes by the International Joint Commission include benzo[a]pyrene (0.01 μg/L).

P. Landrum. I do not know of any.

T. Dillon. (See response to Question B.4).

D. Stainken. There are draft toxicological profiles which have been issued by the U.S. Agency for Toxic Substances and Disease Registry (USPHS) for:

benzo[a]pyrene	dibenz[a,h]anthracene
benzo[a]anthracene	chrysene
benzo[b]fluoranthene	

Each profile presents a review of the International, Federal and State regulatory standards for these compounds.

Aside from these documents, we are not aware of any other defined regulatory criteria outside of the listing of these compounds as priority pollutants with specific regulatory NPDES discharge criteria.

J. Stein. I am unaware of any defined regulatory criteria for sediment for any of the 15 PAHs.

C. Rice. I am not aware of any defined regulatory criteria for any of the 15 PAHs.

R. Spies. I am unaware of any such regulation.

Question B.7. How much sediment will be needed for PAH testing in the proposed tiered testing approach?

R. Peddicord. I wouldn't think PAH testing would require much different amounts of sediment than are now required for present testing approaches.

J. Petty. Several hundred grams of homogenized sediment.

M. Mac. Tier I testing may require 1 gallon of sediment. Tier II testing may require 10 gallons of sediment.

P. Landrum. The amount of sediment required will depend in part on the organism chosen for testing but several kilograms should be adequate.

D. Stainken. The amount of sediment needed for testing will vary with the number and type of chemical tests and bioassays. Generally, at least 100-500 g of sediment should be available for extraction and analysis.

J. Stein. The amount of sediment needed for the chemical analyses would be in the range of 100 grams or less. It is certainly more difficult to estimate the amount of sediment needed for the biological tests since the specific design of the bioassays was not discussed in the first workshop. However, clearly several kilograms of sediment would be required and the actual amount will depend mainly on the number and size of animals to be tested.

C. Rice. I would say that about 20 g would be needed for proper analysis; and 1-kg for the acute toxicity tests and another 1-kg for the bioaccumulation studies.

R. Spies. The amount of sediment required would vary depending on the test and organism, but most sampling grabs and wide-diameter cores would provide sufficient sediment. Otherwise take another.

Question B.8. Can you specify analytical methodology for sediment and tissue analysis that currently can be used by contract laboratories not having research-level capabilities?

R. Lee. For routine analysis of sediment and tissues, high performance liquid chromatography (HPLC) for specific PAH offers some advantages over GC-MS. Reversed phase columns with detection by ultraviolet-visible of fluorometric detections are generally used. Several studies have demonstrated the usefulness of HPLC in analysis of marine sediments and tissues for PAH (Dunn 1980, Lee et al. 1981; Obana, Hori and Kashimoto 1981). A number of methods have been used to extract PAH from sediments. These generally use soxhlet extraction and have included several solvents including toluene-methanol azeotropic mixture (3:7), methanolic KOH, benzenemethanol (1:1), acetone and methylene chloride (Bieri et al. 1978; Prahl and Carpenter 1982; Wakeham, Schaeffner and Giger 1980; Griest 1980).

J. Petty. Those used by the USFWS and to a lesser extent the USEPA.

P. Landrum. The analysis should be performed by GC-MS and capillary chromatography. References abound for PAH analyses in the Battelle symposium volumes on PAH and several should be tried to determine which is best for your use. There is even an EPA method, which was evaluated by Nowicki, Kieda and Bassett (1980).

D. Stainken. I have summarized available methods and the published method detection limit (MDL) for the 15 listed PAH. Table C-3 lists the PAH compounds, the methods and MDL. The methods are either published in the Federal Register as 40 CFR parts 136 (Method 610, 625) or published by the EPA Office of Solid Waste (OSWER) as part of the 3rd edition of SW846 (Methods 8250,

8270, 8310). In Table C-3, the determination of the practical quantification limit (PQL) for the SW846 methods (Method 8310, 8250) is accomplished by multiplying the specific factor listed under each method per matrix by the MDL listed above. The SW846 procedures also present several different extraction procedures. Generally, the target MDL of interest and extraction and sample preparation procedures would need to be specified.

The effects of extraction on recovery and consequent method MDL need resolution. In addition, the target levels of concern (i.e. 0.08 ppb - 0.15 ppb for possible NOEL of some PAH) may require extraction and analysis of bioassay sediments and test organisms which are close to or at the MDL. Many labs may not be able to routinely achieve the MDL without extensive calibration and use of adequate quality control procedures as specified in 40 CFR 136/141 and 142 or SW846.

J. Stein. NOAA's National Analytical Facility, a part of the Environmental Conservation Division, NWAFC, has developed protocols for chemical analysis of sediment and tissues for PAHs and CHs (chlorinated hydrocarbons), including QA/QC criteria (Macleod et al. 1985), that a contract laboratory should be able to use, provided there is documented evidence of an ability to perform such analyses. An interlaboratory comparison was recently conducted using the above methodology, and the results have been submitted for publication (Macleod, Friedman and Brown 1988). Moreover, a HPLC method was recently developed (Krahn et al. 1988) that minimizes the number of organic-solvent extraction steps and gravity-flow column chromatography steps required during extraction of sediments and tissues.

C. Rice. Enclosed is a copy (see Attachment 1) of our current analytical methodology (Gay, Belisle and Patton 1980). We are presently considering altering our method to apply the NOAA Status and Trends Method, which involves sephadex column chromatography and avoids the KOH digestion step in our method.

R. Spies. I would recommend the methods we currently are using (based on Ozretich and Schroeder 1986) for extracting and clean-up of sediments prior to analysis by GC and GC-MS. These methods in the hands of a reasonably well-trained chemist can yield detection limits of from less than one to several ng/g (dry) for PAH. The principal advantage of this extraction and clean-up method is that it employs disposable reverse-phase, silica-gel columns instead of hand-packed columns. This is enormously time-saving. Also, the separation of compound classes appears to be much more efficient than with the traditional silica-gel columns. The only difficulty with the disposable columns is that they leach a few phthalic acid esters. These generally do not interfere with the analyses, however. This procedure has intercalibrated well for sediment PAH with the methods prescribed by the NOAA Status and Trends program. The NOAA data base is very large and covers all coasts and it is advantageous to the Corps that their data sets be comparable to NOAA's.

Question B.9. What specific biological tests would you recommend as tools for assessing toxicity and other adverse biological effects? What species would you use?

R. Lee. The most widely used toxicity test for most compounds are determination of LD50. The LC50 of fluorene, phenanthrene, fluoranthene, benz[a]an-

thracene, chrysene and benzo[a]pyrene is reported to be between 0.3 to 2 mg/liter for various aquatic animals (Neff 1979).

Recent work tested fractions, e.g. PAH fraction, of sediment extracts with regards to their toxicity and mutagenicity by the Ames assay (Reilly, O'Connor and Boone 1986). A variation of this test is to inject sediment extracts into fish and then use liver homogenates of this fish as the activating system when using the Ames test (Kurelec et al. 1979). In other recent work, cataracts noted in croakers collected from the Elizabeth River, Virginia, appeared to be correlated with high PAH concentrations (Huggett, Bender and Unger 1987). We have found that benzo[a]pyrene in the diet resulted in cataract development in these fish (Lee, unpublished data). Other biological tests for PAH are discussed in my response to Questions C.13 and C.14.

R. Peddicord. To test for toxicity and sublethal biological effects of dredged material I would expose organisms to deposited sediment. I would use a polychaete and an amphipod that were deposit feeders or filter feeders at the sediment-water interface. These lifestyles have maximum exposure to sediment associated contaminants. These species have some ability to metabolize PAHs, and it is the metabolites, rather than the parent compounds, that are often the toxic agents. I would look at bioaccumulation from deposited sediment in a bivalve mollusc. Since they have little ability to metabolize PAHs they should retain parent compounds in their tissues. While the parents are of less direct toxicological importance, they are much more easily analyzed than the metabolites.

J. Petty. Acute or partial chronic Daphnia, "worms," fathead minnows (or a species endemic to the area of concern).

M. Mac. Because of the number of PAH compounds, their complexity and potential interactions, regulatory testing for PAH contamination in sediment should stress biological testing. It does not appear conceivable that realistic chemical criteria can be established for a number of PAH compounds. Regulatory decisions will have to be made based on bioassessment. Bioassessment can be conducted through either a series of laboratory tests or through a combination of laboratory and field tests. Specific laboratory tests should involve several organisms measuring several end points such as:

a) Zooplankton toxicity and reproduction test (*Ceriodaphnia*)
b) Benthic invertebrate life cycle (*Chironomus*)
c) Invertebrate bioaccumulation (bivalve or *Pontoporeia*)
d) Carcinogenicity/mutagenicity (see response to Question B.11)

Laboratory tests can be combined with field assessments such as benthic community structure and/or a fish tumor survey to look at the influence of PAH contamination in sediments. The sediment quality triad of Chapman (1986) is another useful application of combined field and laboratory testing.

P. Landrum. In the Great Lakes, *Pontoporeia hoyi* would be useful for bioaccumulation tests and may well be useful for acute and subacute testing. We are currently working with *P. hoyi* to develop an acute test along the lines of the test used by R. Swartz for *Rhepoxynius*.

T. Dillon. Growth/reproduction, driven at least in part by regulations and your concerns.

D. Stainken. It would be logical to use a series of tests similar to those currently in use by the Corps in which a slurry, and a microcosm with the sediment are employed for testing. The problem is that an acute toxicity test may not be sensitive enough to discover toxic effects. Many organisms used for testing are evaluated when they have matured beyond neonate or initial developmental stages. As an example, *Mercenaria* is a very hardy clam from juvenile to adult. More sensitive bivalves would be deposit feeders such as *Macoma*, *Nucula*, *Yoldia*, etc. It is generally assumed that the egg, larvae, molting or settling stages are physiologically different in sensitivity vs. testing young adults. In addition, temperature and varying oxygen content may facilitate toxic effects in the actual environment. Recognizing that regulatory tests cannot evaluate <u>all</u> potential toxic effects, a series of tests might be recommended to include additional items. When toxicity is suspected, a relatively quick (2-4 week) chronic test might be used. The test would be:

a. *Daphnia* chronic 28 day test (using *pulex* or *magna*)
b. Fish egg fry (% development or hatching) (minnows or trout)

Both types of tests have been semistandardized for use in various TSCA and FIFRA testing regimes for environmental hazard assessments. These tests would need further standardization for Corps use but might offer confirmatory evaluation of sediment toxicity.

J. Stein. The biological tests used for assessing the toxicity of sediment should use whole sediment or organic-solvent extracts of the sediments and should employ organisms that will provide a range of response. The use of whole sediments or organic-solvent extracts is stressed, because "elutriate-type" tests are probably meaningless in assessing toxicity of sediments. In our laboratory we have successfully used an amphipod/sediment bioassay (see references in Plesha et al. 1988), using a sensitive species (*Rhepoxynius abronius*), and the bacterial bioluminescence (Microtox) bioassay, using organic-solvent extracts to assess acute toxicity of sediment. The advantages of an amphipod bioassay are that 1) it uses whole sediment, 2) benthic amphipod species are available, and 3) there is literature on its use in Puget Sound, WA, and on factors that influence its effectiveness in screening sediments for the presence of xenobiotic chemicals at toxic levels. An advantage of the Microtox test is that sufficient data can be collected rapidly, at relatively low cost, to allow for statistical evaluation of differences in sediment toxicity. The usefulness of the Microtox test for comparing and ranking the toxicity of organic-solvent extracts of contaminated sediments has been demonstrated (Schiewe et al. 1985). Moreover, the results from this study showed a highly significant relationship between the degree of contamination with aromatic hydrocarbons and toxicity.

Several of the PAHs on the list are known carcinogens in laboratory mammals and fish. Additionally, high prevalences of a variety of lesions, including neoplasms, have been documented in selected bottomfish residing in contaminated urban waterways. Moreover, the PAHs, benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene have been shown to induce hepatic neoplasms in fish (Schultz and Schultz 1982, Hendricks et al. 1985). Thus, an evaluation of the potential chronic toxicity of the sediments should be considered. Recently, an evaluation of the genotoxicity of organic extracts of sediment from the Black River, OH, was conducted (West et al. 1988). The results indicated that a polycyclic aromatic compound (including PAHs) -containing fraction accounted for most of the toxicity of the crude extract when assessed using the Ames

test and an assay for induction of unscheduled DNA synthesis in primary rat hepatocyte cultures.

C. Rice. Based on our studies here at the Patuxent Wildlife Research Center showing that Mallard eggs were sensitive indicators of toxicity of selected PAHs, I would recommend an embryotoxicity test of this type.

R. Spies. I would recommend two levels of testing of contaminated sediments for sublethal effects. The first is a biochemical response of bottom-dwelling fish: induction of P-450E in liver. This P-450 isozyme is induced by PAH and related compounds and will provide a dose-response measurement for accumulation of this class of compounds at environmentally realistic levels. While the consequences of induction for organism fitness are not yet fully appreciated they are clearly implicated in carcinogenesis in some fish species and in reproduction of starry flounder. For example in sexually mature starry flounder captured in San Francisco Bay, one inducible enzyme activity, aryl hydrocarbon hydroxylase, is inversely related to several measures of reproductive success (Spies and Rice, in press). So here is an easily measureable biochemical response in one fish species that responds to environmentally realistic levels of aromatic compounds and relates to reproductive fitness. There is also a very large toxicological literature on chemical carcinogenesis in mammals indicating that induction of P-450 by xenobiotic compounds results in altered lipid and steroid metabolism and chemical lesions on DNA.

The second level of testing is a reproductive assay. This needs to be validated for PAH effects. One of the currently used life-cycle or partial life-cycle tests would seem to be appropriate. Perhaps one of the tests with a polychaete or crustacean would be useful.

Question B.10. In which specific phase should each biological test be conducted (sediment, water column, pore water)?

R. Lee. I believe tests should be carried out in all three phases, i.e. sediment, water column, pore water.

R. Peddicord. Regulatory tests of dredged material should focus on whole sediment. Exposures in the water column are localized and transient. Pore water extractions can introduce artifacts, and the universal importance of pore water as an exposure route is not fully demonstrated.

J. Petty. Sediment and water column. While the pore water would by a useful adjunct, if the proper species is chosen the sediment test should provide data pertinent to this phase.

M. Mac. Whole sediment is the phase which needs to be tested. Only by testing whole sediment will realistic conditions of particulate phase/soluble phase occur. Also, the need exists to test benthic feeders in a situation where dietary input can be considered.

P. Landrum. The tests should be run in sediment, since biological accumulation from sediment seems to be driven by desorption rates from the sediment matrix.

T. Dillon. Again, the decision is driven by your area of concern.

D. Stainken. The biological tests as currently conducted appear adequate in testing slurries and solid phase. In specific cases, depending on the test species and sediment, an elutriate should be tested. As an example, a "greasy" sediment might be better tested by mixing/slurrying and then testing the elutriate rather than trying to keep it in suspension for a slurry test.

J. Stein. Please see the response to Question B.9.

C. Rice. The phase best suited to these tests would be the sediment. A method using direct painting of the sediment onto the egg could be employed. Another approach could be to use a crude extract and paint this on eggs. Either of these would have to be researched before it could be utilized.

R. Spies. Solid-phase tests are most important relative to the environment, especially for sediments that will be managed for containment. For dispersal sites the suspended-phase tests should also be done.

Question B.11. Is it possible to relate the results of short-term laboratory tests to impacts from long-term exposures in the aquatic environment?

R. Lee. Presumably, the long-term effects of interest are those that impact growth and reproduction. Thus, the relationship between the MFO system, reproduction and PAH exposure is one attempt to extrapolate laboratory results to long-term effects. Also, morphological effects at the cellular or subcellular level of reproductive tissues may be extrapolated to reproductive effects.

R. Peddicord. It is clear that adverse effects in a short-term test indicate a real concern. It is possible, but less certain, that the absence of effects in sensitive organisms that have PAH metabolic capability may be cause for little concern.

J. Petty. In my opinion, this is unlikely unless truly adverse effects manifest themselves.

M. Mac. It will be extremely difficult to protect the aquatic environment to any extent unless short-term laboratory tests can be somewhat predictive of long-term exposure. Potential carcinogenicity is perhaps one of the most difficult long-term effects of PAH to predict with short-term testing. In vitro mammalian bioassays have not done a good job of predicting mammalian carcinogens (Tennant et al. 1987), but at this point there is no effective replacement. Several fish assays (trout embryo injection, medaka, guppy) are developing but take appreciable testing time (6 mos. - 1 yr).

P. Landrum. Not at this time.

T. Dillon. Not now.

D. Stainken. Based on the nature of the Corps tests as currently conducted and the list of 15 key PAHs, I do not believe this can easily be done. This question really should be restated as:

What is the regulatory objective?

1. To regulate/permit sediment disposal and dredging?

2. To avoid acute toxicity?
3. To avoid chronic or any toxic effects?
4. To avoid impacting or further reducing the use of fish and shellfish resources through bioaccumulation problems?
5. To avoid increasing mass loadings of contaminants into receiving areas beyond the area assimilative capacity?

Most of the 15 key PAHs are relatively water insoluble and may not exert marked acute toxic effects. However, many of these PAH do exert a chronic effect at less than or equal to ppb levels and can present a potential for bioaccumulation. Therefore, a possible scenario is that a sediment could contain the 15 PAH, not be markedly toxic and present a chronic toxicity and bioaccumulation potential. In addition, these concerns would change when considering upland sediment disposal where the potential leaching of PAH into groundwater would be of prime concern.

J. Stein. Extrapolating the results of short-term acute toxicity tests to predict adverse effects from long-term exposure of aquatic species to chemical contaminants is inappropriate, because acute toxicity and long-term toxicity are generally quite different mechanistically. However, because in mammalian systems short-term tests of genotoxicity (e.g. mutagenicity) have shown a degree of reliability in discriminating carcinogens from noncarcinogens, it may be very fruitful to determine, if such relationships also can be shown in aquatic species.

C. Rice. I would think relating short-term exposures, such as proposed above, to long-term impacts would be difficult and very conjectural.

R. Spies. We do not yet have sufficient information to determine if bioassays are surrogates for chronic long-term effects or what the exact relationship is between short-term bioassay results and long-term chronic effects.

Question B.12. What specific QA/QC procedures can you recommend for sediment analysis or for biological testing?

R. Peddicord. The QA/QC procedures used routinely in sediment analysis should be employed. The most important points in biological testing are the use of a proper reference sediment and a proper grainsize control sediment.

J. Petty. Sediment analysis: performance evaluation materials, methods blanks, blind QC samples, spikes, and matrix spike duplicates, check solutions, and calibration standards.

D. Stainken. There are a series of specific QA/QC procedures which directly apply for the chemical analyses of the proposed 15 PAH compounds in sediments and tissue analyses. These procedures are specified in the publication series of the methods and the methods themselves as follows:

Method 610, 625: 40 CFR Part 136
Method 8250, 8270, 8310: SW846

In addition, there is an EPA guidance document titled "Quality Assurance and Quality Control (QA/QC) for 301(h) Monitoring Programs: Guidance on field and laboratory methods," May 1986, EPA Contract No. 68-01-6938 Final Report, Marine Operations Divisions.

I have reviewed the document titled "Guidance for performing tests on dredged material to be disposed of in ocean waters - NY District, 21 December 1984." The document does require most of the minimal essentials for QA/QC procedures. However, it would be useful if the procedures were itemized more clearly as to what specific requirements will be required rather than referencing multiple documents. As an example, the NJ Department of Environmental Protection (NJDEP) uses an approach in which labs are certified (and receive regulations specifying QA/QC procedures as well as QA/QC procedures within the certified methods) and the programs receiving data from certified labs specify a data deliverable package including QA/QC data which can be evaluated to determine compliance (Stainken 1986).

Several specific requirements that I would suggest to improve QA/QC procedures would be to establish a QA manual specifying:

1. Chain of custody procedures
2. Sampling procedures: i.e. for sediment PAH concentrations, extract triplicate samples
3. Analytical procedures: establish standards, baselines and actual standards library; conduct spike and recovery analyses; determine % recovery, and lab MDL; determine precision, accuracy and bias of method
4. Specify reporting requirement and QA data deliverables
5. Establish mechanism for sending performance evaluation samples of 15 key PAH to labs to gauge lab practice when conducting assays
6. Establish an on-site audit check list procedure to evaluate adequacy of records and adherence to QA procedures. (Reporting of QA procedures after a test doesn't always mean all procedures were followed).

To facilitate review of the QA practices for analytical procedures, the NJDEP uses two basic data deliverable requirements termed Tier I (most extensive) and Tier II. These are itemized in Table C-4.

J. Stein. With regard to sediment analyses the following QA/QC steps should be used: 1) a standard extraction protocol, 2) internal standards, 3) blanks, 4) spiked blanks, and 5) a standard reference material. For biological testing it would be appropriate to use at least the following QA/QC steps: 1) a standard protocol, 2) native sediment(s) (i.e. sediments in which test species are commonly found), 3) standard reference sediments (both contaminated and uncontaminated) tested with the test sediments of interest.

C. Rice. For sediment analyses I would recommend adherence to the NOAA Status and Trends QA/QC guidelines, which basically require use of internal standards, frequent standardization, a regular running of check samples, involvement in frequent round-robin check samples and NBS reference standards, and strict adherence to a prescribed method. I would also recommend analyses by capillary chromatography coupled to a mass spectrometer operated in the SIM mode. I am not qualified to address the issue of QA/QC requirements for biological testing.

R. Spies. I would recommend coordination with the NOAA/NBS Program for measurements of PAH in sediment. There is no comparable program of which I am aware that coordinates quality control in bioassays. There have been some in-

tercalibration exercises with the marine amphipod *Rhepoxynius abronius* (Mearns et al. 1986).

C. Biological Effects of PAH

Question C.13. Please list any information you have concerning specific levels of any of the 15 PAHs that can be related to specific adverse biological effects in specific organisms.

R. Lee. Perhaps the most studied effect of PAH is their ability to induce toxification/detoxification systems. The mixed function oxygenase (MFO) systems of fish and other aquatic invertebrates are induced by PAH (Moore et al. 1987, Lee 1981, Stegeman 1981). MFO activity and P-450 content in fish liver and kidney increase after exposure to PAH (Addison et al. 1979, Gruger et al. 1977, Statham et al. 1978). A large increase in MFO activity was found in fish collected from an oil spill site (Kurelec et al. 1977). Laboratory studies have shown the production of certain form of liver cytochrome P-450 in fish after exposure to PAH (Klotz, Stegeman and Walsh 1983, 1984; Williams and Buhler 1984). The polychaete, *Nereis virens*, collected from a harbor containing sediment high in PAH had six times the MFO activity as worms from a "clean" reference site (Fries and Lee 1984). Worms from the oiled areas lacked or had undeveloped gametes.

In addition to effects of PAH in MFO systems and reproduction, there is subcellular and cellular pathology which has been described in molluscs exposed to PAH. In a number of mollusc species, exposure to oil-derived PAH produces abnormally enlarged lysosomes (Moore et al. 1987). Also, lysosomal stability is affected by exposure to anthracene and phenanthrene, resulting in the release of lysosomal enzymes into the cytosol. This release is believed to increase cell damage and possibly cell death.

Most of the above work was done with PAH in water and dose-response curves were not generated.

Stein et al. (1986) have shown that extracts of urban sediment with high PAH concentrations can affect reproductive success of English sole (*Parophrys vetulus*). A related observation in mammals is the work of Mattison (1980) who found that a number of polycyclic aromatic hydrocarbons destroyed primordial oocytes in mouse ovary and the rate of oocyte destruction was proportional to the ovarian mixed function oxygenase (MFO) activity. Exposure of fish to PAH can affect adrenal and testicular steroidogenesis (Hansson, Rafter and Gustafsson 1980; Truscott et al. 1983).

The presence of induced cytochromes P-450 or increased MFO activity in aquatic animals does not necessarily lead to reproductive impairment. There may need to be an "overloading" of the detoxification system by PAH before reproduction is affected. Further work is necessary to demonstrate what PAH concentrations result in these effects.

Another topic which needs consideration is that the PAH is often not the active toxic compound but a metabolite, e.g. diol epoxide, produced by the MFO system. The accumulation of PAH by mussels is partly due to their limited ability to metabolize PAH.

R. Peddicord. See response to Question B.4 concerning fluoranthene toxicity data and SLCs for several compounds.

T. Dillon. At the general state-of-the-art, making generalizations from published information on residue/effects is exceedingly hard. Analysis and reporting of data will have to become more standardized first before residue/effects generalizations can be made. What we can do is site specific. If a concern is raised in an individual situation, literature can be reviewed and interpreted in terms of specific animals and specific PAHs.

J. Stein. Simple listings of specific levels of PAHs in relation to adverse biological effects can lead to inappropriate inter-study comparisons and interpretations as to environmental significance for the following reasons. It is generally very difficult to make inter-study comparisons of relationships between biological effects measurements and tissue/sediment concentrations of PAHs because of the lack of compatibility, especially in the chemical data. This is primarily due to major differences in extraction and analysis procedures, some of which are semi-quantitative in nature. Thus, a broad synthesis of data is not possible. Moreover, in many laboratory studies a high dose of a PAH is used because the emphasis is on assessing whether a PAH produces a biological effect or on the mechanism of induction of a specific biological change. The use of a high dose that is usually not environmentally realistic makes the results of doubtful environmental significance, because of the inability to make credible extrapolations over, at times, several orders in magnitude in concentration.

Question C.14. Briefly and in general, how do the biological effects of PAHs differ, qualitatively and quantitatively, among different groups of organisms? Do the species recommended in response to Question B.9 differ from each other in sensitivity to PAHs?

R. Lee. (See response to Question C.13)

R. Peddicord. The least effect generally occurs in species having the least well developed capability to metabolize PAH. Exposure to sediment-associated contaminants is maximized in species that feed at the sediment surface or are deposit feeders. Polychaetes and amphipods provide an optimum combination of exposure and metabolic capability, and are most likely to show toxicity. Bivalve molluscs have reasonable exposure to sediments, but low metabolic capability. They generally show low toxicity, but are good indicators of bioaccumulation since the parent compounds that are easily analyzed accumulate in their tissues.

In summary, knowledge of biological effects associated with specific concentrations of sediment-sorbed individual PAH compounds is limited at present. However, ecological effects are not likely to be deducible from "pooled" measurements like total oil and grease, etc., and individual compounds are likely to provide the most interpretable data in the future. Let's start analyzing for the 15 compounds and develop a data base on "typical" dredged materials. Results can be interpreted by comparison to carefully selected reference sediments until a basis for determining effects of particular PAH concentrations is developed. As such information becomes available, it can be incorporated into the evaluative guidance. Development of regulatory guidance for evaluating PAH in dredged material will be an itera-

tive process over several years. Let's begin with what we have now and refine the process as we get new information.

J. Petty. The response of organisms to contaminants is species dependent. Yes, the species recommended in response to Question B.9 will surely differ from each other in sensitivity to PAH intoxication.

M. Mac. Qualitatively, invertebrate species may be more susceptible to acute lethality from PAH contamination and they may also be more sensitive to chronic, particularly reproductive, effects. Because of their metabolic constraints, invertebrates should be used for any bioaccumulation testing. The real threat of PAH to fish remains in the carcinogenic response.

P. Landrum. The types of responses vary with the exposure duration and the mechanism of response changes from an acute narcotic action to a genetic effect. Embryos of sea urchins seem to be susceptible to benzo[a]pyrene in an aqueous exposure.

T. Dillon. Molluscs--low to none metabolic activity; aquatic invertebrates--variable metabolic activity; aquatic fish--generally higher metabolic activity; mammals--very efficient metabolic activity.

J. Stein. There is considerable data on biological effects of PAHs, especially petroleum hydrocarbons, in the literature, and thus it is difficult to briefly summarize the differences observed between species. However, a major factor controlling the types of chronic effects induced by PAHs in organisms both from different phyla and within the same phylum, or even within the same family appears to be their ability to metabolize PAHs, both in a qualitative and quantitative sense (Varanasi 1988).

R. Spies. The data available to aquatic toxicologists generally indicates that organisms that most efficiently metabolize PAH will be most susceptible to PAH effects. Also, chronic low-level exposure to such compounds is more of a problem than acute toxicity at concentrations that occur in most contaminated areas.

Table C-1. PAH Compounds in Great Lakes Sediment and Biota

Acenaphthene *	Fluoranthene *
Acenaphthylene *	Fluorene *
Acetylnaphthalene	Indeno(1,2,3-cd)pyrene *
Anthracene *	1-Methylanthracene
Benz(a)anthracene *	9-Methylanthracene
Benzo(b)fluoranthene *	Methylbenzanthracene
Benzo(k)fluoranthene *	1-Methylnaphthalene
Benzo(b)fluorene	2-Methylnaphthalene
Benzo(d,e,f)fluorene	1-Methylphenanthrene
Benzo(g,h,i)perylene *	5-Methylphenanthrene
Benzo(a)pyrene *	Naphthalene
Benzo(e)pyrene	1-Naphthylamine
Decahydro-2,3-dimethylnaphthalene	Perylene
Decahydronaphth[2,3-b]oxirene	Phenanthrene *
Dibenz(a,h)anthracene *	Phenylnaphthalene
1,2-Dihydro-2,5,8-trimethylnaphthalene	Pyrene *
1,3-Dimethylnaphthalene	

From: Great Lakes Water Quality Board, 1987, 1987 Report on Great Lakes Water Quality, International Joint Commission, Windsor, Ontario. Annex - 1986 Working List of Chemicals in the Great Lakes Basin, p. 230.

* Recommended for inclusion in regulatory evaluation of dredged material (see Workshop Proceedings)

Table C-2. Correlation Matrix[a] (Pearson's r) for PAH in San Francisco Bay Sediments (TOC-Normalized); Unpublished Data for Approximately 40 Stations

	Benzo (a) Pyrene BaP	Anthra-cene AN	Phen-anthrene P	Methyl-phen-anthrene MP	Fluor-anthene FLUO	Py-rene PYR	Benz-(a)an-thracene BaA	Chry-sene CHRY
BaP	1							
AN	0.979	1						
P	0.956	0.968	1					
MP	0.756	0.812	0.807	1				
FLUO	0.78	0.821	0.827	0.976	1			
PYR	0.741	0.794	0.796	0.984	0.991	1		
BaA	0.87	0.908	0.879	0.942	0.932	0.931	1	
CHRY	0.92	0.956	0.965	0.91	0.917	0.906	0.949	1
BbF	0.931	0.956	0.981	0.837	0.848	0.837	0.9	0.981
BkF	0.853	0.888	0.894	0.8	0.733	0.743	0.857	0.903
BeP	0.985	0.986	0.957	0.815	0.826	0.804	0.912	0.952
PERY	0.631	0.664	0.697	0.744	0.69	0.699	0.701	0.724
BPERY	0.883	0.859	0.879	0.745	0.806	0.778	0.799	0.863

	Benzo(b)-fluor-anthene BbF	Benzo(k)-fluor-anthene BkF	Benzo-(e)-pyrene BeP	Pery-lene PERY	Benzo-(g,h,i)-perylene BPERY
BbF	1				
BkF	0.915	1			
BeP	0.957	0.886	1		
PERY	0.705	0.727	0.664	1	
BPERY	0.879	0.691	0.895	0.64	1

[a]Correlation indicates the degree of relationship between two variables, and ranges from -1 (perfect inverse relationship) to +1 (perfect direct relationship), with 0 indicating no relationship.

Table C-3. PAH Analytical Methods for Sediment and Tissue Analysis. Methods Designed for Analysis of Clean Extracts Generally Extracting 1000 ml of Sample and Analyzing 1 ml of Extract

	Method Detection Limits (ng/L)			
	HPLC 610	625 GC/MS	GC/MS 8250	HPLC 8310
Acenaphthene	1.8uv	1.9	1.9	1.8uv
Acenaphthylene	2.3uv	3.5	3.5	2.3uv
Anthracene	0.66	1.9	1.9	0.66
Benz(a)anthracene	0.013	7.8	7.8	0.013
Benzo(b)fluoranthene	0.018	4.8	4.8	0.018
Benzo(k)fluoranthene	0.017	2.5	2.5	0.017
Benzo(g,h,i)perylene	0.076	4.1	4.1	0.076
Benzo(a)pyrene	0.023	2.5	2.5	0.023
Chrysene	0.15	2.5	2.5	0.15
Dibenz(a,h)anthracene	0.030	2.5	2.5	0.030
Fluoranthene	0.21	2.2	2.2	0.4
Fluorene	0.21uv	1.9	1.9	0.21uv
Indeno(1,2,3-cd)pyrene	0.043	3.7	3.7	0.043
Phenanthrene	0.64	5.4	5.4	0.64
Pyrene	0.27	1.9	1.9	0.27

Determinations of Practical Quantitation Limits (PQL) in SW846

Matrix	Factor 8310	Factor 8250
Groundwater	10	10
Low level soils sonification with GPC	670	670
High level soils sonification with GPC	10,000	10,000
Non-water miscible waste	100,000	100,000

Table C-4. New Jersey Department of Environmental Protection (NJDEP) Quality Assurance Requirements Within NJDEP Tier I and Tier II Data Packages

Tier I
(Used for NJDEP CERCLA Projects and/or Special Projects)

1. Title page
2. Sample analysis request form
3. Chain of custody record (with sample shipment container)
4. Chain of custody record
5. Laboratory chronicle
6. Methodology summary
7. Targeted analyte - summary of quantitative results
8. Water matrix spike/matrix spike duplicate recovery
9. Soil matrix spike/matrix spike duplicate recovery
10. GC/MS tune summary: volative organics
11. GC/MS tune summary: extractable organics
12. Initial calibration data: volative organics
13. Initial calibration data: extractable organics
14. Continuing calibration check: volatile organics
15. Continuing calibration check: extractable organics
16. GC/MS surrogate recovery data
17. Non-targeted analyte summary
18. Pesticide/PCB standard summary
19. Pesticide/PCB identification
20. Analytical results and quality assurance data: metals
21. Initial and continuing calibration verification: metals
22. ICP interference check sample summary
23. Method of standard addition results
24. 2,3,7,8-TCDD data report form
25. 2,3,7,8-TCDD partial scan confirmation
26. 2,3,7,8-TCDD initial calibration summary
27. 2,3,7,8-TCDD continuing calibration summary

Tier II
(Modified Reporting Format for Routine NJDEP Analytical and Monitoring Work)

1. Chain of custody record
2. Sample request form
3. Methodology summary
4. Laboratory chronicle
5. Organic analyses by GC/MS (volatiles, acid and base/neutral extractables) to include tune summary with signature certification, quantitative results and quality assurance data
6. Surrogate compound recovery summary
7. Sample total ion chromatogram (TIC)
8. Pesticide/PCB (GC/ECD), analysis and QA data to include method blank, spiked blanks, matrix spikes, tune summary with signature, etc.
9. Metals analysis to include method detection limits and method blank results

NOTE: Both the Tier I and Tier II reports must be bound and paginated in a legible format.

Attachment 1

Analytical Method for PAH from "Patuxent Analytical Manual" [SOP]
Environmental Residue Chemistry Section
1987
U.S. Fish & Wildlife Service
Patuxent Wildlife Research Center
Laurel, MD 20708

PE 1.0 Oil

PREPARATION AND EXTRACTION OF TISSUE SAMPLES

1.1 Reagents
1. Solvents
   a. Petroleum ether, Burdick & Jackson
   b. Methylene chloride, Burdick & Jackson
   c. Hexane, non-spectro grade, Burdick & Jackson
   d. Ethyl ether, Burdick & Jackson
2. Acetic acid, Fisher, reagent ACS
3. Potassium hydroxide, pellets, certified ACS, Fisher
4. Sodium sulfate, anhydrous, granular, reagent, ACS, MCB

1.2 Apparatus
1. Centrifuge tube, Pyrex, 50 ml
2. Balance, Mettler
3. pH meter, Fisher, Accumet model 230A
4. Flask, flat-bottom, Pyrex, 300 ml, 24/40 joint
5. Separatory funnel, 500 ml, Pyrex
6. Evaporator, rotary, Buchler Instruments
7. Extraction heater, Lab-line, multi-unit
8. Beaker, Pyrex, 400 ml

1.3 Tissue Preparation
1. Tissue Samples - Tissue is ground or cut up into small pieces. When analyzing fat, 2 g are used, eggs require 5 g, and other tissues 15 g. Following homogenization, sample is placed in a 50 ml centrifuge tube.
2. Procedural blanks - For each set of samples, at least one procedural blank will be run. The blank is initiated with saponification and treated through the whole process exactly as a sample.

1.4 Saponification - To the centrifuge tube is added potassium hydroxide solution (6 N, 25 ml). The tube is tightly capped with a ground glass stopper, sealed with Parafilm, and placed in a constant temperature bath at 35°C for 24 hours. For the first seven hours the reaction mixture is agitated every hour.

1.5 Neutralization - The centrifuge tubes are removed from the constant temperature bath and chilled in an ice-water bath. The alkali solution is neutralized with acetic acid (glacial, 15 ml). Care is required to add the acid slowly with shaking and simultaneous cooling to avoid overheating the solution.

1.6 Extraction - To a 500 ml separatory funnel is added 100 ml of distilled water. The saponification mixture is then added and the centrifuge tube is rinsed with deionized water and methylene chloride. The rinsings are added to the separatory funnel and the aqueous solution is extracted three times with 25 ml of methylene chloride. The organic layer is drained into a 300 ml flat-bottomed

flask. The aqueous layer is discarded and the organic layer is returned to the separatory funnel. The organic layer may be cloudy. It is extracted with potassium hydroxide solution (2 N, 150 ml), and the aqueous layer is repeatedly back-extracted with methylene chloride (25 ml). The methylene chloride extracts are combined in the flat-bottomed flask.
The methylene chloride extract should at this point contain all the hydrocarbons, along with other lipophilic compounds from the tissue.

CO 2.0 Oil

CONCENTRATION OF SOLVENTS

2.1 Apparatus
1. Flash evaporator, Buchler Instruments Co.
2. Flat-bottom flask, 300 ml
3. Pasteur/capillary disposable pipettes fitted with large rubber bulb

2.2 Method - The solution is placed in a flat-bottom flask, iso-octane (2-3 ml) is added, and the volume is carefully reduced to 5-8 ml on the flash evaporator. A flocculent precipitate may form at this point. It may be removed by filtering the methylene chloride solution through about 1 g of anhydrous sodium sulfate. The sodium sulfate must then be thoroughly rinsed with methylene chloride. Petroleum ether (100 ml) is then added to the methylene chloride solution and the volume is again reduced to 3-5 ml. This procedure removed the methylene chloride. The sample may now be transferred as required, depending on the next step in the analysis procedure.

CU 3.0 Oil

FLORISIL CLEANUP

3.1 Reagents
1. Solvents
   a. Petroleum ether, Burdick & Jackson
   b. Ethyl ether. Burdick & Jackson
2. Sodium sulfate, anhydrous granular, Baker
3. Florisil, 60-100 mesh, Floridin Company

3.2 Eluting mixture
6% ethyl ether in petroleum ether

3.3 Preparation of Florisil
Preparation of Florisil and anhydrous sodium sulfate is described in the organochlorine section of the Patuxent Analytical Manual.
To prepare the Florisil column, add 21 g Florisil to column with gentle tapping, use mark on column. Top column with 1/2" Na_2SO_4.
Measure out 200 ml eluting mixture.
Prewash Florisil column with 100 ml petroleum ether. When the hexane has just reached the top of column, replace receiving vessel with Phillips beaker and add sample to top of column. Aliquot should not contain more than 0.5 g of lipids. Let sample sink into column and immediately rinse down sides of glass column with 3 x 2-ml portions of eluting mixture. Allow each portion to sink into top of column. Never allow the column to go dry. Add remainder of eluting mixture to column.

Evaporate sample down to about 3 ml on a flash evaporator as described in CO 2.0.

SG 4.0 Oil

SILICIC GEL COLUMN

4.1 Reagents
1. Silica gel, Davison Chemical, grade 923, mesh size 100-200 ASTM, special for column chromatography
2. Solvents
   a. Petroleum ether, Burdick & Jackson
   b. Methylene chloride, Burdick & Jackson

4.2 Apparatus
1. Chromatographic column, 400 x 22 mm id with 24/40 outer joint, coarse fritted plate, and Teflon stopcock (Kontes # 420550, C-4) with addition funnel, 500 ml, with Teflon stopcock, 24/40 inside joint on stem, and 24/25 outside joint at top (Kontes # 633030).
2. Receiving flasks, 300 ml, 22/40 flat-bottom flasks
3. Tube, 10-ml concentrator, graduated, Kontes # 570050-1025

4.3 Eluting solvents
1. Petroleum ether
2. Methylene chloride:petroleum ether::2:3

4.4 Preparation of silica gel. Silica gel is prepared exactly as described in the organochlorine section of the Patuxent Analytical Manual.

4.5 Procedure

Weigh 20 g of silica gel in 250 ml Erlenmeyer flask and immediately slurry with 80 ml petroleum ether; pour slurry into column with stopcock open, rinsing flask and side of column with small portions of petroleum ether. Use a total of 100 ml petroleum ether. Tap the column with handle of spatula and allow the petroleum ether to drain out. When the petroleum ether is about 3 mm above the surface of the silica gel (never allow column to go dry) close stopcock and discard solvent.

Place 250 ml receiver under column to collect the aliphatic fraction. Transfer cleaned up sample from flask onto column. Add the sample slowly and touch the tip of the pipet to the side of the column so as not to disturb the top of the silica gel. Rinse flask with several 1 ml portions of petroleum ether and add to column. Open stopcock until solvent level is 3 mm above the silica gel. Rinse sample onto column with 3 x 2 ml petroleum ether, draining each 2 ml aliquot to 3 mm above surface of column. Close stopcock, carefully pipet 10 ml of petroleum ether on top of the glass column, open stopcock, and obtain an elution rate of approximately 5 ml/min.

Continue elution until petroleum ether is 3 mm above silica gel. Close stopcock and change receiving flasks.

Add 100 ml of polar eluting mixture (40% methylene chloride in petroleum ether to funnel. Pour some of the mixture slowly down the sides of the column, open stopcock and continue eluting until polar mixture is 3 mm above silica gel. Close stopcock. Elute the most recalcitrant PAHs with an additional 50 ml methylene chloride. Combine polar eluates. To avoid problems with the column "breaking up" it is necessary to elute very slowly.

Concentrate the aliphatic and aromatic fractions as described under CO 2.0, and transfer to 10-ml concentrator tubes. Samples are now

ready for analysis by GC and/or GC/MS.

GC 5.0 Oil

GAS CHROMATOGRAPHY

5.1 Apparatus

1. Hewlett Packard Model 5840 gas chromatograph (GC) equipped with an FID detector, model 18740 glass capillary inlet system.
2. Pasteur pipets, 230 mm
3. Syringe, Hamilton 701, 10 ml capacity. Check volume to be injected with magnifying glass.

5.2 Instrument and Column Parameters

1. Gas chromatograph
   a. Detector temperature 300°C
   b. Injection port temperature 250°C
   c. Chart drive 0.25 in./min.
2. Column conditions
   a. Gas - helium thru column, nitrogen for make-up
   b. Flow rates:
      Helium carrier gas thru column: 1.5 ml/min (tank = 80 psi)
      Total carrier (w/N make-up): 51 ml/min (N tank = 40 psi)
      Hydrogen: 39 ml/min (gas inlet at 18.2 psi)
      Air: 240 ml/min (gas inlet at 50 psi)
   c. Column
      25 mm x 0.75 mm od, 0.25 mm id, cross-linked fused silica, with coating of SE-54.
   d. Column temperature
      Initially 40°C for 2 min then increased to final temperature of 265°C at 4°C/min; run time approximately 1.5 hours.
   e. Inlet purge activation time
      Splitless injection mode = 50 sec.

5.3 Procedure

Adjust the volumes of the 10 ml tubes from the silica gel separation. For typical injection, draw 1 μl of solvent, and 2 μl of sample containing known weight of reference standard (e.g., 40 ng C D). Individual hydrocarbons are measured by integration of area. Amounts found may be based on an internal standard (ISTD) or external standard (ESTD) calibration. Quantitations by ISTD are preferred when (1) relative response factors are reproducible, and (2) there is no sample interference with GC measurement of the reference peak. ISTD calibration is performed by the addition of a constant amount of the standard (40 ng) to a specified volume (1 ml) of a mixture containing a known amount (e.g. 12 ng) of each compound under investigation. The solute concentration is chosen near that which exists in the unknown sample. A response factor is calculated for individual solutes relative to the response factor (amt./area) for the internal standard (1.0). The actual analysis is performed by adding the same amount of the internal standard to 1 μl of the unknown mixture. The amount of unknown solute is determined from the following calculation:

$$(\mu g) = (\text{rel. response factor} \times \text{area}) \times (\text{dilution factor}) \times 40 \text{ ng}$$
$$(\text{area})_{ISTD}$$

MS 6.0 Oil

GAS CHROMATOGRAPHY/MASS SPECTROMETRY

6.1 Apparatus
1. Finnigan 3200 Gas Chromatograph/Mass Spectrometer
2. Finnigan INCOS 2300 C Data System

6.2 Capillary Column Parameters
1. Gas - helium
2. Flow rate - 4.5 μl/min
3. Column - 25 m cross-linked, fused silica, SE-54
4. Inlet - Grob injector

6.3 Instrument and Column Parameters
1. Gas chromatograph
   a. Injection port - 250°C
   b. Injection at room temperature. After 2 min. program initiated. 160 to 200°C at 2°/min to 200°C.
2. GC/MS Interface: direct transfer line heated to 240°C.

6.5 Procedure

Sample eluates are concentrated to 1 ml. Ten μg of the internal standard are added to the sample, and 1 μl of the solution is injected, using the Grob inlet. Data acquisition is begun immediately. The Grob injector is a device which permits selective venting of the solvent. It therefore prevents solvent tailing and permits injection of relatively large volumes on a capillary column. It is used as follows:
1. Cool column to room temperature. Close vent.
2. Inject sample. High molecular weight samples condense on column. After 20 sec. open vent, flushing solvent.

CO 7.0 Oil

REMOVAL OF CONTAMINANTS

The following precautions must be used to reduce background levels of hydrocarbon contaminants.

7.1 Procedure
1. Glassware - all detergent-washed glassware is subjected to additional cleaning to remove hydrocarbon contaminants. Glassware may be effectively cleaned in chromic acid or "Micro" solution, followed by solvent rinsing. Handling chromic acid can be hazardous. Instead, we use "Micro" solution (International Products Corp., P.O. Box 118, Trenton, N.J.). The glassware is placed in an ultrasonic tank (Sonicor, Randall Mfg. Co., Hillside, N.J., model TSI2046) containing 2% Micro solution and cleaned ultrasonically for 15 minutes and allowed to soak for three hours. The glassware is then thoroughly rinsed with distilled water, acetone, and methylene chloride.
2. Sodium sulfate - heat in muffle furnace at 675°C for 3 hours.

CUG 8.0

PAH CLEANUP

The procedure for the analysis of polynuclear aromatic hydrocarbons is identical to that for oil, with the following exceptions:

1. Fractionation on silica gel preceeds the florisil step.
2. Florisil cleanup is applied only to the aliphatic fraction.
3. The aromatic fraction is cleaned up by gel permeation chromatography as described below.

8.1 Reagents

1. Bio-Beads (SX-12), 200-400 mesh, catalog number 152-3650, Bio-Rad Laboratories, Rockville Center, NY.
2. Solvents
   a. Methylene chloride, Fisher "pesticide grade"
   b. Cyclohexane, Burdick & Jackson, "Distilled in glass"

8.2 Apparatus

1. GPC AutoPrep 1001, Analytical Biochemistry Laboratories, Inc., Columbia, MO.
2. Chromatographic column, 600 x 25 mm id, equipped with plunger assembly for organic solvents, catalog # K-422351-6025, Kontes Glass Company, Vineland, NJ.
3. Syringe, 10 cc Luer-Lok (B-D Cornwall) with special needle, 6" guage 20 (B-D #1364) and Millipore swinny filter 13 mm #239-910), ABC Laboratories.
4. Flask, flat-bottom, 250 ml, 24/40 T joint
5. Filter paper, 5.0 μm pore size, 13 mm dia., PTFE, (LSWP 013 00), Millipore (optional).

8.3 Eluting Solvent

1. Methylene chloride cyclohexane (1:1 v/v)

8.4 Column Preparation

1. Weigh 80 g of Bio-Beads resin into a 800-ml beaker and add sufficient eluting solvent to permit swelling of the resin.
2. Insert bottom plunger assembly allowing approximately 45 cm between it and the top plunger.
3. Using the eluting solvent mixture, quantitatively transfer the resin to the column.
4. After the resin has settled and excess solvent has drained from the bottom of the column, position the top plunger of assembly and connect column to GPC.
5. Pump solvent through column for 4 hours; if cracks or air spaces develop, loosen one plunger and compress column.
   CAUTION: DO NOT exceed 15 psi; leaks may develop in tubing connections or pressure gauge may be damaged.

8.5 Pumping Rate Adjustment

A 5.0 ml per min. pumping rate is normally maintained for PAH residue cleanup.

8.6 Operation

A 10-ml glass syringe is used to manually inject the sample into the instrument. Each sample loop holds 5.0 ml of sample solution; however, it is necessary to inject a minimum of 7 ml to fill loop and eliminate cross contamination.

1. Turn on instrument exhaust fan.
2. Disconnect GPC column from system and join inlet and outlet lines together from pump. Seal column by connecting top and bottom lines together from pump.
3. Switch loading valve to "OPERATE" position with dump, collect and rinse settings of 1-2 minutes for all sample loops to be loaded.
4. Press "pump enable" and "auto start"; allows solvent to flush air from each sample.
5. After air is evacuated, reconnect inlet and outlet lines to column. If column contains air, inlet lines should first be

connected to top of column, outlet line to bottom.

6. Pump solvent through column until most of air in column has been evacuated.
7. Reverse inlet and outlet lines to column, so that inlet line is now connected to bottom of column.
8. Resume pumping solvent through column until air is completely evacuated from column.
9. Switch chromatographic cycle to "standby."
10. Reset the sample loops to "00."
11. Open sample loading valve to the "load position."
12. Fill the zeroeth loop with eluting solvent and index to the next ("01") loop.
13. Shake sample tube thoroughly and draw 7 ml into syringe using attached filter to exclude coarse particulate matter. The use of filter paper inserted in the swinny filter is recommended for samples with fine particulate matter, this will prevent clogging of the column. When the filter paper is used, it will take more time to fill the syringe.
14. Detach needle and filter from syringe; slowly inject sample solution through load valve.
    CAUTION: Excessive pressure from syringe while loading sample may result in damaged seals and sample loss.
15. While the syringe is still affixed to the instrument, index to the next sample loop "02." Detach syringe and remove filter paper if used; rinse syringe, filter, and needle with clean solvent; dry and repeat procedure until all samples have been injected into the instrument. Rinse the loop following the last sample with clean solvent.
16. Close sample loading valve mechanism.
17. Set the "terminal sample loop" to the same number as the number of loops that are loaded.
18. Set "dump", "collect", and "wash" all to 30 minutes.
19. Switch to "pump enable"; let pump operate for several minutes to check column for air spaces.
20. Place effluent lines in 250 ml collection flasks.
21. If no air spaces appear on column, switch to "auto start" to begin GPC cleanup.

APPENDIX D: SCOPES OF WORK

Long-Term Effects of Dredging (LEDO) Program
Work Units Involving PAH

PROGRAM TITLE: Long-Term Effects of Dredging

PROGRAM MANAGER: Robert Engler (601) 634-3624

TECHNICAL MONITOR: Robert Pierce (202) 272-0199

PROBLEM: In the early 1970's, concern over the environmental effects of dredging operations reached the stage where Federal legislation mandated the CE to undertake a major study to determine the environmental effects of dredged material disposal and to develop procedures for minimizing any adverse effects. The 5-year (1973-78) Dredged Material Research Program (DMRP) was completed and provided the first definitive information on impacts of dredged material disposal. Due to the short term duration of the DMRP, long-term effects were not addressed. The CE is, however, legislatively required to evaluate, assess, and minimize long-term effects of dredged material disposal.

OBJECTIVE: The principal objectives of LEDO are to provide new or improved technology to predict long-term (including cumulative) environmental impacts and to address methods of minimizing any adverse impacts. The technology will allow the CE to meet its dredging and regulatory missions in a manner that is environmentally sound while reducing or eliminating unneeded environmental constraints imposed on these activities by other agencies. Development of state-of-the-science assessment technology is essential in the planning, design, construction, and operation of CE dredging projects as well as in evaluating permitted activities.

DESCRIPTION OF WORK: The LEDO program work units are grouped into two general environmental impact areas: (1) effects of aquatic disposal, and (2) effects of upland disposal. Development of first generation predictive tests for: (1) determining bioaccumulation and consequences in aquatic organisms/plants, (2) techniques for predicting leachate and effluent quality from CDFs, and (3) relationships between sediment geochemistry and biological impacts.

ACCOMPLISHMENTS: Refinement of rapid method for predicting final bioaccumulation potential in aquatic organisms continues to receive broad peer and interagency approvals. The consequences of bioaccumulation of toxic metal contaminants on the reproductive success of selected aquatic organisms resulted in development of a mechanism for estimating contaminant residues from exposure concentrations. Lab and field studies on capping provided techniques to chemically and biologically isolate contaminated dredged material. Prediction of contaminant uptake by plants continues for freshwater and saltwater species.

PROGRAM TITLE: LONG-TERM EFFECTS OF DREDGING

WORK UNIT #: 31772 PRIORITY: 03

WORK UNIT TITLE: Toxic Substances Bioaccumulation in Aquatic Organisms

PERFORMING LAB: WES PRINCIPAL INV: Mr. V. A. McFarland (601) 634-3721

PROBLEM: Legislation for dredged and fill material discharges pursuant to Sections 103 and 404 of the Ocean Dumping and Clean Water Acts, respectively, requires bioaccumulation evaluations at or beyond the present state of the art. In order to select the preferred disposal alternative and to minimize conflicts, delays, and litigation, predictive methods for determining bioaccumulation of toxic substances from dredged and fill material are essential.

OBJECTIVE: To finish development of a reliable, rapid and cost-effective method for predicting body burden of persistent, common contaminants in fresh and saltwater organisms. To investigate sources of variability in estimation procedures and to recommend standard analytical techniques. To develop algorithms for bioaccumulation potential estimation of the most commonly encountered chemicals in sediments and to incorporate these into a computerized evaluation system.

DESCRIPTION OF WORK: Primary rate-incluencing variables and determination of the partitioning coefficients and kinetic terms describing processes will be evaluated. Laboratory exposures, field validation of hypotheses and computer searches of relevant case studies are being employed. Analytical methods for lipid and organic carbon determinations will be standardized. Bioaccumulation of selected PAH by non-metabolizing aquatic biota will be considered. Prototype software incorporating bioaccumulation assessment techniques and using Artificial Intelligence and Knowledge-based System techniques will be developed.

ACCOMPLISHMENTS: A series of experiments assessing the interactions and independent influences of major environmental variables on the uptake of heavy metals in clams and fish was conducted. Preliminary investigations in the bioavailability of metals and PCBs from suspended contaminated sediments were completed and are being analyzed. The thermodynamic bioaccumulation potential (TBP) hypothesis was tested and applied. HPTLC/microbial analytical techniques were successfully applied to PAH-contaminated sediments. A second prototype knowledge-based computer program was developed.

MILESTONES:

	Scheduled Completion Date
MP D-86-5: "Changing Concepts and Improved Methods for Evaluating the Importance of PCBs as Dredged Sediment Contaminants"	8607
MP D-89-2: "Preliminary Recommendations for a Congener-Specific PCB Analysis in Regulatory Evaluation of Dredged Material"	8707
TN EEDP-01-14: "Influence of Environmental Variables on Bioaccumulation of Mercury"	8809
TN EEDP-01-17,18,19,20: "Factors Influencing Bioaccumulation of Sediment-Associated Contaminants by Aquatic Organisms"	8909
Draft Guidance Manual - Predicting Bioaccumulation	9009

TECHNOLOGY TRANSFER: Non-Mission Related Technology Transfer Potential--An application assessment of the potential for successful transfer of the technology or data resulting from this work unit to state and local governments and to private industry, in accordance with Public Law 96-480, has been performed. The assessment indicates that a product resulting from this work unit has high potential for non-mission technology transfer.

PROGRAM TITLE: LONG-TERM EFFECTS OF DREDGING

WORK UNIT #: 31773 PRIORITY: 02

WORK UNIT TITLE: Environmental Interpretation of Consequences of Bioaccumulation

PERFORMING LAB: WES PRINCIPAL INV: Dr. T. M. Dillon (601) 634-3922

PROBLEM: The Ocean Dumping and Clean Water Acts and subsequent regulations governing the discharge of dredged and fill material require that environmental consequences of bioaccumulation be evaluated. Presently, there is little interpretive guidance on whether or not the predicted level will result in environmental impact. This work unit will reduce project delays caused by regulatory debate over this issue by providing data on biological consequences of particular levels of bioaccumulation.

OBJECTIVE: To determine and document levels of metals, organohalogen compounds and Polynuclear Aromatic Hydrocarbons (PAH) bioaccumulated from dredged material causing adverse effects on reproduction and survival potential of important fresh and saltwater organisms.

DESCRIPTION OF WORK: The biological consequences of bioaccumulation will be studied in aquatic animals exposed to a variety of important organohalogen and metallic contaminants as well as PAHs. Correlations of bioaccumulation with the biological parameters of reproduction and survival potential will be emphasized in a variety of representative aquatic organisms. These parameters will be assessed in freshwater and saltwater animals. The magnitude of change in key parameters which causes deleterious effects to the organisms will be investigated and correlated to the degree of tissue contamination. Through this approach results of mandated bioaccumulation studies can be realistically interpreted.

ACCOMPLISHMENTS: Contractual and in-house research on the ecological importance of individual PCB congeners was presented at 5 scientific meetings. The scientific literature pertaining to the biological consequences of bioaccumulation of organohalogen contaminants and heavy metals by marine organisms was summarized in a draft TN (Dec 88). A Journal Article was submitted for publication describing the consequences of PCB congener bioaccumulation in *Daphnia magna*. Experiments establishing the quantitative relationship between tributyl tin and PCB tissue concentrations and reproduction in marine worms (*Neanthes arenaceodentata*) have recently been concluded.

MILESTONES:

	Scheduled Completion Date
TR D-84-2: "Biological Consequences of Bioaccumulation in Aquatic Animals: An Assessment of the Current Literature"	84
MP D-85-2: "Bioaccumulation and Effects on Reproduction in Aquatic Organisms: An Assessment of the Current Literature"	85
TN EEDP-01-6: "Computerized Database for Examining the Relationship Between Contaminant Tissue Residues and Biological Effects in Aquatic Organisms"	87

TN EEDP-01-7: "The Relationship Between Mercury and Cadmium Bioaccumulation and Survival, Growth, and Reproduction in the Freshwater Crustacean, *Daphnia magna*"	87
TN EEDP-01-13: "Relationship Between PCB Tissue Residues and Reproductive Success of Fathead Minnows"	8812
Journal Article (submitted to Environmental Toxicology and Chemistry): "Effects of Selected PCB Congeners on Survival, Growth, and Reproduction in *Daphnia magna*"	8909
Draft TN: Effects of Petroleum Hydrocarbon Bioaccumulation in Aquatic Animals	9009
Input to EPA/CE Implementation Manuals	CONT

TECHNOLOGY TRANSFER: Non-Mission Related Technology Transfer Potential--An application assessment of the potential for successful transfer of the technology or data resulting from this work unit to state and local governments and to private industry, in accordance with Public Law 96-480, has been performed. The assessment indicates that a product resulting from this work unit has high potential for non-mission technology transfer.

PROGRAM TITLE: LONG-TERM EFFECTS OF DREDGING

WORK UNIT #: 32571 PRIORITY: 01

WORK UNIT TITLE: Relationships Between Sediment Geochemistry and Biological Impacts

PERFORMING LAB: WES PRINCIPAL INV: Dr. J. Brannon (601) 634-3725

PROBLEM: If EPA decides to promulgate sediment quality criteria (SQC) under Section 404 of PL 92-500, CE dredging activities will be evaluated based on SQC to determine environmental impacts of aquatic disposal. Lack of direct involvement in SQC-related research places the CE in an awkward position; such was not the case when previous criteria were promulgated and the DMRP was underway. To substantially contribute to SQC regulations, the CE must have ongoing research into the relationships between sediment geochemistry, soluble contaminant concentrations, and biological impacts, and be a recognized leader in this research area.

OBJECTIVE: Investigate and delineate the factors responsible for the regulation of the bioavailability of contaminants associated with sediment. Determine if contaminant activities measured in sediment affect contaminant bioavailability by examining sublethal toxicity and bioaccumulation.

DESCRIPTION OF WORK: Using lab studies, determine the impacts that sediment properties responsible for adsorption and release of sediment contaminants such as PCBs, PAHs, and metals have on soluble contaminant concentrations and bioavailability. Factors responsible for regulating the bioavailability of contaminants in sediment (carbonates, sulfides, organic carbon, and iron oxides) will be investigated for sediments of varying physical and chemical characteristics. Results will demonstrate the relationship between sediment contamination, sediment physical and chemical properties, and sediment pore water concentrations and impact on biota, either through sublethal toxicity or bioaccumulation.

ACCOMPLISHMENTS: New Work Unit.

MILESTONES:

	Scheduled Completion Date
Draft TN: "Development of Procedures for Examining the Relationship Between Sediment Geochemistry and Biological Impacts"	8909
Draft MP: Interim Results--Effects of Sediment Organic Matter Composition on Relationship Between Sediment Geochemistry and Biological Impacts	9009
Draft TN: Influence of Sediment Properties on Bioaccumulation Potential	9109
Draft MP: Interim Results--Effects of Metals Associated with Sediment	9209
Draft TN: Evaluation of Metal Bioavailability from Sediment	9309
Draft MP: Interim Results on Interactive Effects of Sediment Properties	9409
Draft MP: Sediment Geochemistry and Biological Effects of Interactive Contaminants	9509
Draft TN: Final Guidance	9609

TECHNOLOGY TRANSFER: Non-Mission Related Technology Transfer Potential--An application assessment of the potential for successful transfer of the technology or data resulting from this work unit to state and local governments and to private industry, in accordance with Public Law 96-480, has been performed. The assessment indicates that a product resulting from this work unit has high potential for non-mission technology transfer.

APPENDIX E: PROPOSED PAH STUDY
UNDER THE WATER QUALITY RESEARCH PROGRAM

PROBLEM: The environmental and water quality effects of petroleum hydrocarbons are not quantitated at the present time. Development of testing criteria for petroleum hydrocarbons in both marine and freshwater is needed for regulatory purposes Corps-wide.

PRODUCT(S) DESIRED: A testing criteria for petroleum hydrocarbons in marine and freshwater using a tiered testing approach, the fifteen priority pollutant PAHs and appropriate bioassay/bioaccumulation organisms as identified in the Proceedings of the New York District/Chicago District Petroleum Hydrocarbons Workshops. This would include development of a database for bioaccumulation of the 15 PAHs as indicative of levels of concern for petroleum hydrocarbons in marine and freshwater.

ASSESSMENT OF THE PROBLEM: Petroleum hydrocarbons are ubiquitous in Hew York/New Jersey Harbor sediments as well as in industrial port areas nationwide. Development of testing criteria for petroleum hydrocarbons is of particular concern now, considering the recent Alaskan oil spill. There is a real danger that without timely development of effects-based petroleum hydrocarbon criteria, the Corps may be forced by USEPA to use a non-technically based numeric sediment criteria for petroleum hydrocarbons. This sort of criteria applied across the board would cause economic hardship and possibly the closing of some ports. In addition, it is likely that major oil companies will commence drilling for oil off North Carolina within the next year. Preliminary modeling of an oil spill off North Carolina indicates that some of the material would probably be carried onshore along the East Coast by the Gulf Stream. These considerations make addressing the need for technically based petroleum hydrocarbons criteria essential.

PAST COORDINATION: The Ocean Dumping Criteria and the USEPA/Corps Guidance specify that the levels of trace contaminants in dredged material must not cause unacceptable adverse biological impacts or that potential impacts be rapidly rendered harmless if the material is to be ocean disposed. In 1979, USEPA Region II, New York District and an interagency regulatory group established "matrix" values (limits for uptake of certain chemical constituents in marine organisms). The results of bioassays and bioaccumulation of dredged material were compared with a "clean" reference site and with the "matrix" value to determine its suitability for ocean disposal. At that time, analytical techniques by contract laboratories precluded testing for anything other than total petroleum hydrocarbons and a level of concern ("matrix" value) could not be established for regulatory purposes. The agencies agreed to revisit the "matrix" values at a later time when analytical techniques for isolating petroleum hydrocarbons were available at contract laboratories. Up to this point the only indication of the effects of petroleum hydrocarbons were based on statistical significance with respect to reference sediment and the effects of synergism were not known. Several years later the agencies suggested that the "matrix" values be reconsidered. Similar problems with petroleum hydrocarbon-contaminated sediments were encountered throughout the country. To address this problem, New York District and Chicago District sponsored two workshops on petroleum hydrocarbons which were run by CEWES on a reimbursable basis. These workshops were attended by experts on petroleum hydrocarbons from the United States and Canada. As a result of the workshops, agreement was reached on the approach to be used for determining petroleum

hydrocarbons testing criteria. However, it became apparent that data on the effects of petroleum hydrocarbons and levels of concern as determined by uptake in tissues are virtually unknown for marine systems and sparse for freshwater systems. The second workshop reaffirmed the use of the 15 priority pollutant PAHs as indicators and presented the tiered testing approach within the context of the Federal Standard (POC Dr. Robert Engler, CEWES). Inclusion of these studies in the Long-Term Effects of Dredging (LEDO) Program was suggested last year. There was sufficient interest in them to be added to LEDO, but the funding was insufficient to do any meaningful new start work. Due to budget cuts this funding was redistributed within existing programs. The continuation of this work to benefit all Corps Districts and Divisions is essential and would be appropriate under the Water Quality Research Program due to its applicability Corps-wide and its technical merit. Funding of this research under the Water Quality Research Program would also show that the Corps is responsive to the present and growing concern for petroleum hydrocarbon contamination and oil spills and their effect on water quality and biota. Inclusion of this project in the Water Quality Research Program was discussed with Don Robey (C, CEWES-EL-ERSD) and Thomas Patin (CEWES-EL), both of whom thought that the research proposal had merit on a nationwide basis. Development of a testing criteria for petroleum hydrocarbons is anticipated to take two years (including development of the database for bioaccumulation of PAHs). It is anticipated that the cost per year would be approximately $250K ($500K total). The cost of not developing a technically based criteria for petroleum hydrocarbons and allowing a numerical one to be imposed by USEPA could be billions of dollars. Closure of just one major port, such as the Port of New York and New Jersey, due to the imposition of non-technically based petroleum hydrocarbons criteria and subsequent inability to dredge would cost at least $26 billion.

POCs: John F. Tavolaro, Chief, CENAN-OP-W, (212) 264-5620
Carol A. Coch, CENAN-OP-W, (212) 264-5621